THE UNIVERSITY OF ALBERTA

THE EFFECT OF WAVELENGTH AND INTENSITY OF LIGHT ON THE ENDOCRINE SYSTEM OF THE MOUSE

by

© JERRY VRIEND

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "The effects of wavelength and intensity of light on the endocrine system of the mouse" submitted by Jerry Vriend in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

The sensitivity of the endocrine system of *Peromyscus maniculatus* to light intensity and wavelength, as well as the sensitivity of two inbred strains of the genus *Mus*, was investigated. Pituitary, gonad, adrenal and spleen weights were taken at 60 days, from mice born and reared in light environments of several intensities and several colours. In addition, counts of circulating eosinophils were taken of deer mice at 42 days of age.

The response of mice reared under different light environments depended on sex and strain of animal and on the particular organ studied. Adrenal weight of deer mice was higher in animals under dim light than in darkness, but lower in animals under bright light than under dim light. Eosinophil counts were greater in deer mice under bright light than in animals under dim light or darkness. Spleen weight increased with intensity of light under which deer mice were reared.

Darkness or orbital enucleation inhibited the development of gonads, suggesting that the eye is the photoreceptor for this response. Gonadal enlargement in response to light was intensity-dependent. When deer mice were reared under coloured lights of equal radiant energy those in red light had larger gonads than those in the shorter wavelength blue and green light. This differential response of the gonads to wavelength was lost when the spectral environments were equated as to number of quanta. Thus this research shows for the first time that the relationship between spectral characteristics of the light source and gonadal growth depends on the number of quanta of light to which the animals are subjected.

Inbred strains of *Mus musculus* reach maturity at an earlier age than *Peromyscus maniculatus*. Light also influenced the development of these inbred strains, the response depending on sex and strain. This study revealed differences in sensitivity to light even between closely related genetic lines. Gonad weights of C57/Bl and BALB/c mice reared under dim light were greater than under bright light or darkness. Intensity-dependent differences in adrenal weights were significant only for males. Adrenal weights of males of both strains were greater under bright white light than under dim white light but there were strain differences under higher intensity blue light. Spleen weights for both sexes were greater in mice under bright light than under dim light. The differences in spleen weight were intensity dependent and changes were greater for mice of the BALB/c strain than for the C57/Bl strain. These spleen weight changes suggest involvement of the immune system in the responsiveness of mice to light.

The differences observed in the two species and the two strains suggest genetic differences in sensitivity to hormones during development.

This study has elucidated several aspects of the photoendocrine responsiveness of mice, with respect to the physical parameters of light important in eliciting a response; with respect to light effects during the developmental period as distinct from those in the adult; and with respect to the photoresponsiveness of the spleen as an indicator that the immune system may be intimately influenced by responses of the developing endocrine system to the light environment.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Boreal white-footed mouse

Peromyscus maniculatus borealis

INTRODUCTION

The deer mouse has been extensively studied under a variety of natural and experimental situations. This is a study of the boreal white-footed mouse, *Peromyscus maniculatus borealis*, a subspecies abundant in the aspen parkland and coniferous forest of northwest Canada. These animals spend the winter under the cover of snow without hibernating (Stebbins, 1971). Winter breeding is not common (Fuller, 1969). Some aspect of the environment, presumably light, acts as a stimulus to bring these animals into breeding condition in the spring. Evernden (1966) has observed that the light intensity reaching the sub-nivean environment is controlled by the depth of the snow cover and that those rays which penetrate the spring snow cover are mainly long wavelength (red) rays; he has suggested that the onset of breeding of *Clethrionomys gapperi*, the red-backed vole, is related to the decrease of snow depth and the change of its light transmittance properties in the spring.

It is clear that many rodents are photosensitive, but of the several aspects of the light environment, only the effects of photoperiod have been extensively studied. Very little work has been done on the effects of wavelength and intensity of light on any mammal. The present study was designed to investigate the effects of wavelength and intensity of light on the maturing endocrine system of the boreal deer mouse. The responses of these mice were compared with those of two inbred strains of mice, C57/Bl and BALB/c.

Genetic Considerations

Peromyscus maniculatus is a mammal of considerable genetic diversity. It has been suggested that no two individuals are completely alike in genetic constitution (Rasmussen, 1964). A study of any physiological variable in such a population must recognize that under natural conditions the physiological variable is part of a viable system, the result of genetic integration brought about through natural selection operating on the genetic variability of the population. The choice of *Peromyscus maniculatus* for this study is predicated on the interest in the relationships of such a physiological system to the environment. When studying the influence of any environmental parameter such as light on the maturation of a system as complex as the endocrine system of the mammal, considerable variability as a result of genetic factors might be expected. Parallel study of the responses of two inbred strains of mice of the closely related species *Mus musculus* permits comparison of physiological effects in a genetically viable system of a natural population with specific responses in inbred strains.

Photoresponsiveness Involving the Endocrine System of Birds and Mammals

There is considerable evidence that light influences the activity of endocrine organs and endocrine-regulated systems in various birds. Etzold (1891) and Schafer (1907) suggested that a relationship exists between daylength and seasonal testicular development in birds. Rowan (1925) conducted experiments demonstrating that light serves as an

environmental stimulus regulating events associated with migration and reproduction in birds. When juncos (*Junco hyemalis*), which normally breed in May, were subjected to artificial increases in daily photoperiod, he found that gonadal growth could be induced out of season, in mid-winter. Photoperiodic control of gonadal cycles has since been demonstrated in many avian species (see reviews by Farner, 1959; and Wolfson, 1966).

Bissonette (1930, 1931a) found that, when starlings (*Sturnus vulgaris*) were kept under the same light regime, the testis response depended on the previous light history. Testes of birds with a short-photoperiod history became active under subsequent long photoperiods, and testes of birds with a long-photoperiod history regressed under subsequent treatment with the same long photoperiod. Poultry research workers have found it necessary to distinguish the prior from the immediate light pattern in effects on reproductive system responses (Hutchinson and Taylor, 1957). Abplanalp and Wilson (1960), studying the "aftereffects" of light regimes on egg production of Leghorn chickens, concluded that the response of domestic fowl to light depends on the total amount of light supplied (intensity) as well as on the photoperiod. They found that chickens with a history of total darkness (from age 16 to 40 weeks) responded to a continuous light period (40 to 50 weeks) with a greater rate of egg production than chickens with a history of intermittent light exposure of 1 minute every hour (during the same 16 to 40 week period). The magnitude of the response of the chickens reared in the dark increased with intensity.

Photoperiodic effects on the reproductive system of mature birds can be distinguished from light effects on the maturing reproductive

system. The rate of sexual maturation has been found to be intensity-dependent in a number of species of birds. Bissonnette (1931b) found that spermatogenic activity of starling testes increased with the intensity of light up to a threshold of 180 lux (17.6 ft. c.). Similar evidence was obtained for the English sparrow, *Passer domesticus*, by Bartholomew (1949). He also found that the growth rate of ovaries and oviducts increased with light intensity. However the intensity beyond which no further increase was effective was different for males than for females. Kirkpatrick (1955) presented evidence that the intensity threshold for the testicular response of the bobwhite quail, *Colinus virginianus*, is about 10 lux (.92 ft. c.); the age at first egg for female quail was intensity-dependent up to a maximum of 3200 lux (296 ft. c.).

It has long been known that egg production by the domestic hen can be increased by long photoperiods (Whetham, 1933). Darkness delays sexual maturation in Leghorn chickens (King, 1962). Wilson *et al.* (1956) found that light intensity becomes the limiting factor in sexual maturation of Leghorn chickens when the photoperiod is restricted.

Several experiments have also demonstrated the effects of light on the mammalian reproductive system. Brown *et al.* (1924) studied the seasonal weight changes of several endocrine glands (pituitary, testes and adrenal) and organs of the immune system (thymus, spleen and lymph nodes) in rabbits. Organ weights of rabbits maintained in constant light were lower than those of controls in natural (diurnal) light or in darkness, and showed no seasonal fluctuations. Further evidence that light influences the activity of mammalian endocrine systems comes from the work of Baker and Ransom (1932), who observed almost complete

failure of reproduction in the field mouse, *Microtus agrestis*, when the daily photoperiod was reduced from 15 to 9 hours. Bissonnette (1932a) found that by extending the natural photoperiod with artificial light premature estrus could be induced in the ferret, *Putorius vulgaris*, a seasonal breeder. Marshall (1940) found this response to be intensity-dependent. He placed ferrets at different distances from a white light source and found the acceleration of estrus varied according to distance from the light source. Marshall and Bowden (1936) reported little difference between the effects of a two-hour bright light treatment and a 16-hour light treatment at one-eighth of this intensity. Hammond (1954), who found that a 15-hour photoperiod was more effective in inducing estrus than a 24-hour photoperiod, suggested that the optimum photoperiod for this reproductive response would be found to vary for different intensities of light. The few reports of light effects on the ferret suggest that possibly the light received by this mammal in its natural habitat acts to stimulate the onset of breeding condition as it does in mice and voles.

Since the work of Baker and Ransom (1932) light has been shown to influence reproductive phenomena in various species of both wild and laboratory-bred rodents. Diurnal activity variations associated with estrus in the rat are reversed (peak activity occurs in the dark period) when the day-night pattern is reversed (Hemmingsen and Krarup, 1937). Continuous light causes precocious puberty in the rat (Luce-Clausen and Brown, 1939), prolonged vaginal cornification (Browman, 1937) and eventually leads to an anovulatory "persistent estrus" syndrome (Fiske, 1941; Dempsey and Searles, 1943). The ovarian response to continuous

light in the rat is biphasic, involving first an increase and later a decrease in gonad weight (Fiske, 1941). Logan (1954) found that the period required before development of the "persistent estrus" syndrome decreases with increasing intensities of illumination and with increasing age of the rat. Constant darkness inhibits secretion and release of FSH, delays puberty in the rat and mouse, inhibits gonadal steroidogenesis and spermatogenesis (Desjardins *et al.*, 1971) and causes a decrease in size of reproductive organs of the rat (Fiske, 1941; Itoh *et al.*, 1962) and hamster (Hoffman and Reiter, 1965).

The eyes mediate these endocrine influences of light in the ferret (Clark *et al.*, 1939), rat (Browman, 1938) and hamster (Reiter, 1969). Browman found that removal of the lens of both eyes did not prevent the vaginal cornification response to continuous light, if the retina remained intact. There is also evidence for an extra-retinal photoreceptor for the gonadal response to light. Benoit (1938) has demonstrated that light, focussed on the hypothalamus via a quartz rod, was effective in stimulating the gonads of enucleated ducks (*Anas platyrhynchos*). Ganong *et al.* (1963) implanted a light-sensitive photovoltaic cell in the hypothalamus and temporal lobe of sheep, dogs, rabbits, and rats and found that light can reach the brain directly through the skull. Lisk and Kannwischer (1964) observed vaginal cornification in enucleated rats after focussing light by way of optic fibres directly onto the hypothalamus. It appears, however, that extra-retinal photoreceptors are of more importance in avian than in mammalian photoreception (Menaker, 1971).

Clark *et al.* (1937) found that the pathway by which light information normally reaches the hypothalamus of ferrets to stimulate estrus

requires an intact inferior accessory optic tract, but that the response still occurs in animals whose visual cortex has been removed or in animals in which the optic tracts have been cut. Knoche (1957) has described retinohypothalamic fibres in the rat and Critchlow (1963) has suggested that the influence of continuous light is mediated by fibres which pass directly from the optic chiasma to the hypothalamus.

The pineal gland appears to be involved in regulation of endocrine function under different environmental lighting conditions in the rat (Reiter and Hester, 1966), hamster (Reiter, 1969), ferret (Herbert, 1971) and in most birds studied (see review by Ralph, 1970). Activity of the melatonin forming enzyme, hydroxyindole-O-methyl transferase, in the rat pineal is increased when the animal is exposed to continuous darkness, and is decreased by exposure to continuous light. Moore *et al.* (1967) found that orbital enucleation or inferior accessory optic transection (but not transection of the optic tracts) prevented this response.

In the rat light also affects events associated with the pituitary-adrenal axis, such as the weight of the adrenal (Fiske, 1962) and plasma corticosterone levels (Leeman *et al.*, 1962). Laurens (1928), in a review of the effects of light on several mammals, discussed early experiments showing that maturation of cellular elements in the blood is influenced by long-term changes of the light environment. In darkness, neutrophils increase at the expense of lymphocytes and eosinophils. In light of sufficient intensity and duration lymphocytes and eosinophils increase and neutrophils decrease. There are several reports of variation in weights of lymphoid organs with changes in the light environment. For

example, Brown *et al.* (1924) reported an increase in thymus weight and a decrease in spleen weight of rabbits maintained in continuous light. Wurtman and Weisel (1969) have confirmed that spleen weight is very sensitive to changes in the light environment. Although the role of the pituitary trophic factors in the maturation of blood cells and lymphoid tissue is not well understood, adrenocortical depression of lymphocyte (Dougherty, 1959) and eosinophil counts (Spiers and Meyer, 1949) via ACTH has been well documented.

In investigating endocrine responses to light several researchers have studied the effects of wavelength. Bissonnette (1932b) found that when European starlings were subjected to red or green light of equal luminous intensity (280 lux), testis growth and spermatogenesis occurred only in birds subjected to red light. Dakan (1934) maintained chickens under white, red, yellow or blue light and observed highest egg production under red light. Scott and Payne (1937) reported that white light or light of long wavelengths, provided (from 0430 to 0700) in addition to winter solar light, brought about sexual maturity in turkey hens two months in advance of the normal breeding season. Benoit and Ott (1938) found that when immature male ducks were subjected to various coloured lights of equal luminous intensity (436 lux), the testes enlarged only in ducks subjected to red light. When light was conducted directly to the hypothalamus via a quartz rod, the entire visible spectrum was effective in stimulating testis growth. Ringoen (1942) found that the gonadal response of the English sparrow, *Passer domesticus*, was also greater in red than in green light. Burger (1943) reported that only the part of the visible spectrum between between approximately 580 and 680 nanometers

(yellow through red) induced spermatogenesis in male starlings. Ishibashi *et al.* (1951) reported that in female Leghorn chickens raised under blue, green or red light (5 to 30 lux) sexual maturation was accelerated under the long wavelengths. Carson *et al.* (1958) on the other hand, using various coloured environments of considerable spectral overlap, found that all light treatments, regardless of colour, accelerated the onset of sexual maturity in Leghorn pullets, in comparison with dark controls.

These pioneer studies vary in the extent to which light intensity was controlled. A feature of all these wavelength studies is that intensity was equated (if it was measured at all) in luminosity units (foot candles or lux), units of measurement based on the sensitivity of the average human eye. Radiant energy units of measurement are more suitable for such wavelength studies since radiant energy is a direct measure of light intensity.

Hollwich and Tilgner (1961) found that in male ducks subjected to monochromatic light of wavelength 436, 546, 632 or 707 nanometers and of equal radiant energy (245 $\mu w/cm^2$) for 200 hours only the longer wavelengths were effective in inducing gonadal response. Harrison *et al.* (1970) reported that in Leghorn chickens raised under blue, green, red or white light of maximum intensities .08, .11, .14 or 3.2 $\mu w/cm^2/nm$ respectively, testis growth was least in blue and greatest in white light. Foss and Arnold (1969) designed a more carefully controlled experiment. They found that in broiler cockerels subjected to blue, green, red or infrared light (filters had peak transmittance at 450, 545, 650 and 750 nanometers respectively) of equal radiant energy (80 $\mu w/cm^2$), long

visible wavelengths stimulated reproductive development most effectively. More specifically they found that pituitary weight, gonadotrophin content of the pituitary, comb weight and testis weight were greatest for birds raised under red light, although infrared was not effective.

There are only a few reports concerning the effects of wavelength on endocrine function in mammals. Barbanti (1932) raised white mice under each of five different light conditions described as white, blue, green, yellow and red (no indication of intensities is given) and took data on number of litters, litter size, sex ratio, and body weights for two generations. He also raised two female mice with a male under each of the five light conditions to study the onset of puberty as measured by the birth date of the first litter. From his data Barbanti concluded that red light (and yellow to a lesser degree) acts as a stimulant for reproductive functions (early puberty, greater number of offspring, increased body weight of young). He reported a disproportionate number of female offspring from mothers born and maintained under red light. Blue and green light, Barbanti concluded, had a definite inhibitory action on reproductive functions (retardation of puberty, smaller number of offspring, average body weight lower than that of white controls). A greater-than-expected number of male offspring was born to mice raised in blue and green light. Marshall and Bowden (1934, 1936) reported that the acceleration of the recurrence of estrus in the ferret induced by extra light (8 hours of artificial light after dusk) was affected not only by intensity but also by wavelength. Ultraviolet light was most effective in stimulating the acceleration of estrus and infrared the least. This wavelength experiment, however, was complicated by intensity

differences between treatments. Luce-Clausen and Brown (1939) found that darkness and infrared light inhibited body growth and delayed sexual maturity in the rat; rats raised in diurnal visible light (12 hours of light and 12 hours of darkness--12L/12D) had greater body weights than those raised in darkness or under infrared, and they reached sexual maturity sooner. These investigators also presented data for 3 generations on number of offspring and percentage of young surviving in the three experimental environments. Number of offspring was greater in darkness than in visible light. However, percentage survival of offspring was greater in visible light. Wurtman and Weisel (1969) have shown that organ weights differed significantly between rats raised under two types of fluorescent light sources (differing only in the proportions of the wavelengths emitted): ovary, testis and pineal weights were higher, and spleen weight lower, in "Vita-light" than in "Cool-white" light. There was no difference in rate of body weight gain in these animals. This study differed from previous action spectrum studies in that the number of quanta of light was equal in the two environments. Another significant difference was that the light sources included emissions in the ultra-violet, visible, and infrared rather than a narrow waveband only.

The present study was undertaken to investigate the relative influence of wavelength, energy intensity and number of quanta on the maturing endocrine system of the mouse. Earlier studies indicated that both wavelength and intensity of light do affect the maturation and function of endocrine glands in the mammal, but such studies have not made clear how the separate variables of wavelength, energy intensity and

quanta influence endocrine maturation of the mammal. The purpose of this study was to search for effects of these distinct variables on several photosensitive endocrine parameters of the mouse.

MATERIALS AND METHODS

Source of Animals

Pregnant deer mice were trapped during May and June of 1970 and 1971 in the outlying areas of Ellerslie, St. Albert and Beverly, near Edmonton, Alberta. Sherman* live traps were baited with peanut-butter and rolled oats and set out in the afternoon or evening. Animals were collected the next morning and brought into laboratory animal quarters where they were maintained in standard rodent cages until used for experiments. Pregnant laboratory mice of the BALB/c and C57/Bl strain were obtained from stocks maintained by Bioscience Animal Services of the University of Alberta.

Animal Care

Pregnant deer mice were kept in 6" by 8" by 11" plastic cages with wire tops. They were supplied with Teklad** rat and mouse diet and water *ad libitum*. Before females gave birth to litters they were transferred to environment chambers designed to provide precise experimental lighting conditions. Litters were weaned at 21 days of age and sexes were separated at 30 to 35 days. Litter size varied from 3 to 7.

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*H. B. Sherman, Deland, Florida.

**Teklad Inc., Monmouth, Illinois.

Environment Chambers

Litters were maintained from birth in cages placed in light-tight environment chambers made of plywood. The front of each chamber was supplied with a port designed to give access to the animals and to serve as a light-baffled air vent. Each environment chamber measured 24" by 24" by 16" and contained 4 plastic mouse cages. Temperature was maintained at 25 ± 1.5°C. Ventilation was supplied via an airline through the lid of each chamber. The photoperiod was kept at 18 hours of light and 6 hours of darkness (18L/6D) by means of a time clock.*

The lid of each chamber was fitted with an 11½ inch square plastic or glass filter. Neutral density filters made of smoked clear plastic or window glass were used for experiments with white light. Coloured filters were obtained from Carolina Biological Supply House.** Peak spectral transmittance for these filters was 450 nm (blue), 545 nm (green) and 650 nm (red). Light was supplied by a 150 Watt incandescent flood lamp*** mounted 20 to 30 inches above the filter by means of a metal stand. Cupric sulphate (2 cm deep of a 5% solution in a pyrex container) was used to filter out most of the infrared portion of the spectrum. A photograph of the environment chamber is shown in Figure 7.

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*Paragon poultry timer, Model 41005-0, Paragon Electric Co. Inc., Two Rivers, Wisconsin.

**CBS filter set, A24631, Carolina Biological Supply Co., Burlington, North Carolina.

***Sylvania 150PAR/SP PAR 38, Sylvania Electric Ltd., Montreal, Que.

Light Measurement

Spectral energy intensity was measured with a spectroradiometer* fitted with an optic fibre sensor. The units of measurement express energy flow per unit area per unit bandwidth (microwatts per square centimeter per nanometer). Before use the spectroradiometer was calibrated against a standard lamp.** According to the manufacturer's specifications, the instrument is exceptionally stable and has an accuracy of 7 to 10 percent (depending on the wavelength) after calibration.

Intensity values given (recorded through copper sulphate, filter and wire cage top) are maximum values measured at the center of the environment chamber at the level of the mice. Desired intensity levels were obtained either by changing the distance of the lamp from the filter or by adding neutral density filters.

Experimental Procedure

Experiment 1. Influence of wavelength and intensity of light on organ weights and eosinophil counts of deer mice

An experiment was designed to study the effects of wavelength and intensity of light on organ weight and eosinophil levels of deer mice. Litters were maintained from birth to 60 days of age in six experimental environments: (1) Nine litters were reared in constant darkness (0L/24D); (2) Six litters were reared under "dim" white light (18L/6D) of intensity

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*ISCO Model SR, Instrumentation Specialties Co., Inc., Lincoln, Nebrasca.

**ISCO Model SRC.

16 $\mu w/cm^2$; (3) Eight litters were reared under "bright" white light (18L/6D) of intensity 2250 $\mu w/cm^2$; (4) Eight litters were reared in each of three coloured light environments (18L/6D). The area under the energy transmittance curve was adjusted to be equal (8.4 $\mu w/cm^2$) in the three environments.

Figures 1 and 2 show the spectral distribution of the two white light systems. Figure 3 shows the spectral composition of the light transmitted by each of the coloured filter systems. Since the green filter used had a slightly narrower passband the peak intensity is higher than those for the other two filter systems.

For purposes of comparison with earlier published experiments, luminosity values of the total light energy transmitted by the coloured filter systems were calculated. To convert spectral energy to luminosity requires that energy be multiplied by a conversion factor (0.632) relating light energy to luminosity in foot-candles at a wavelength of 555 nanometers (the maximum sensitivity of the human eye); and also by a luminosity weighting factor (L.W.F.) at the selected wavelength. Multiplication by 10.8 converts foot-candles to lux. The following equation expresses the calculation.

$$\text{Lux} = \mu w/cm^2 \times .632 \times 10.8 \times \text{L.W.F.}$$

To convert total energy under the transmittance curve, energy intensities of increments of 25 nm were converted to luminosity values and summed. Luminous intensity in the blue environment was 6.8 lux; in the green 81.7 lux; and in the red 17.4 lux.

At 42 days of age .05 cc of blood was taken from the tail and

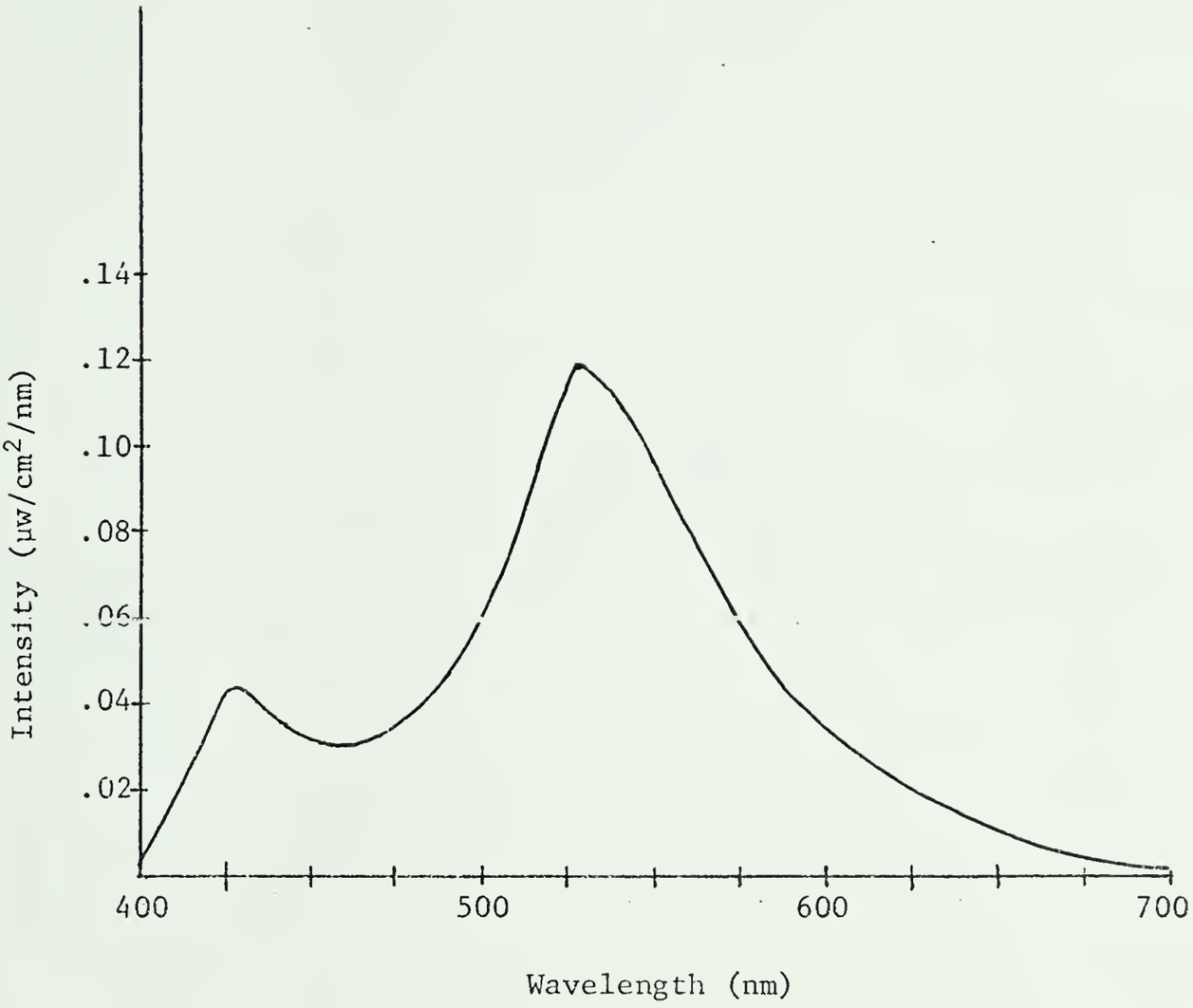

Figure 1. Spectral composition of light transmitted by smoked plastic filter and $CuSO_4$ in Experiment 1. ("Dim light")

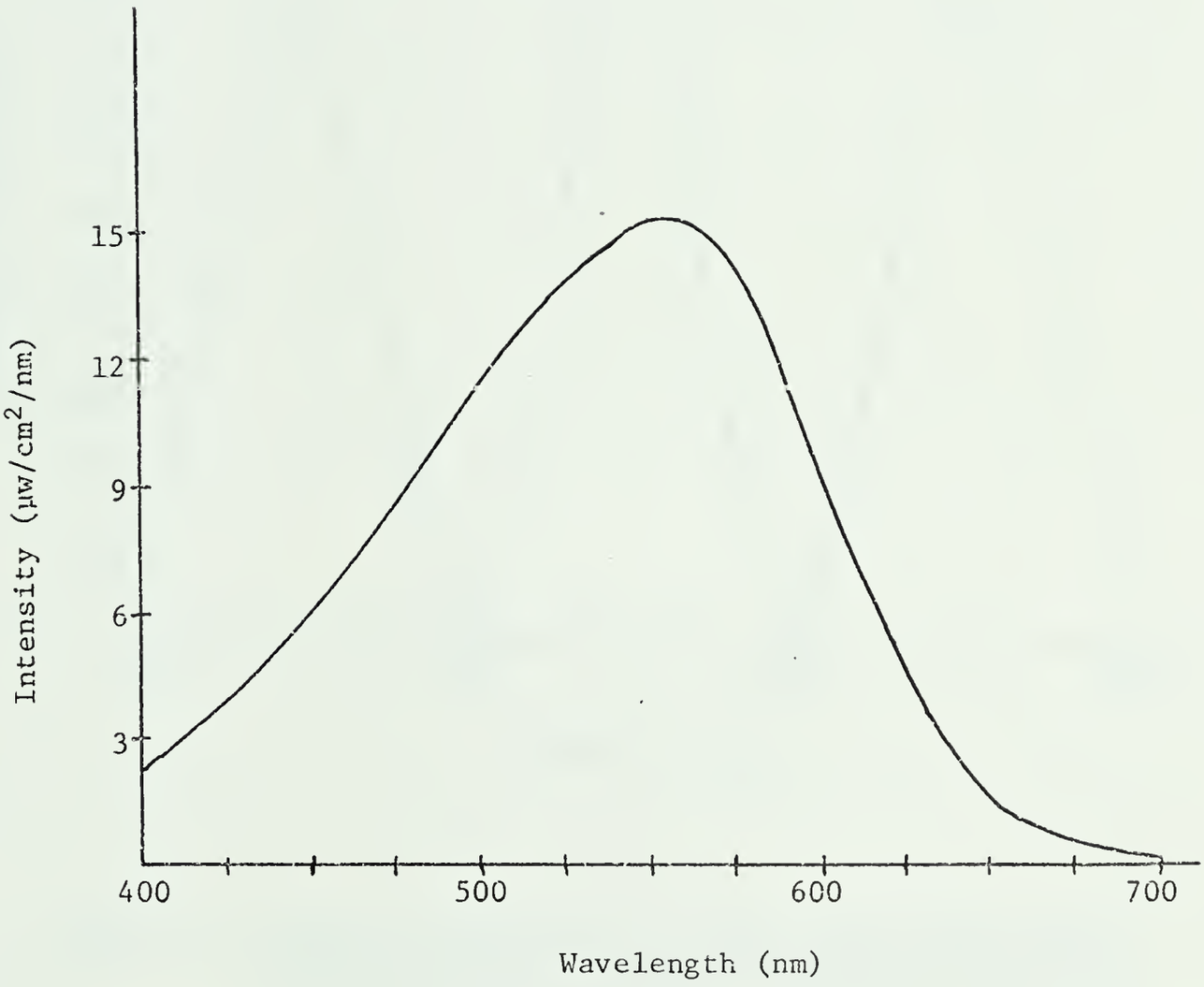

Figure 2. Spectral composition of light transmitted by clear glass filter and $CuSO_4$ in Experiment 1. ("Bright light")

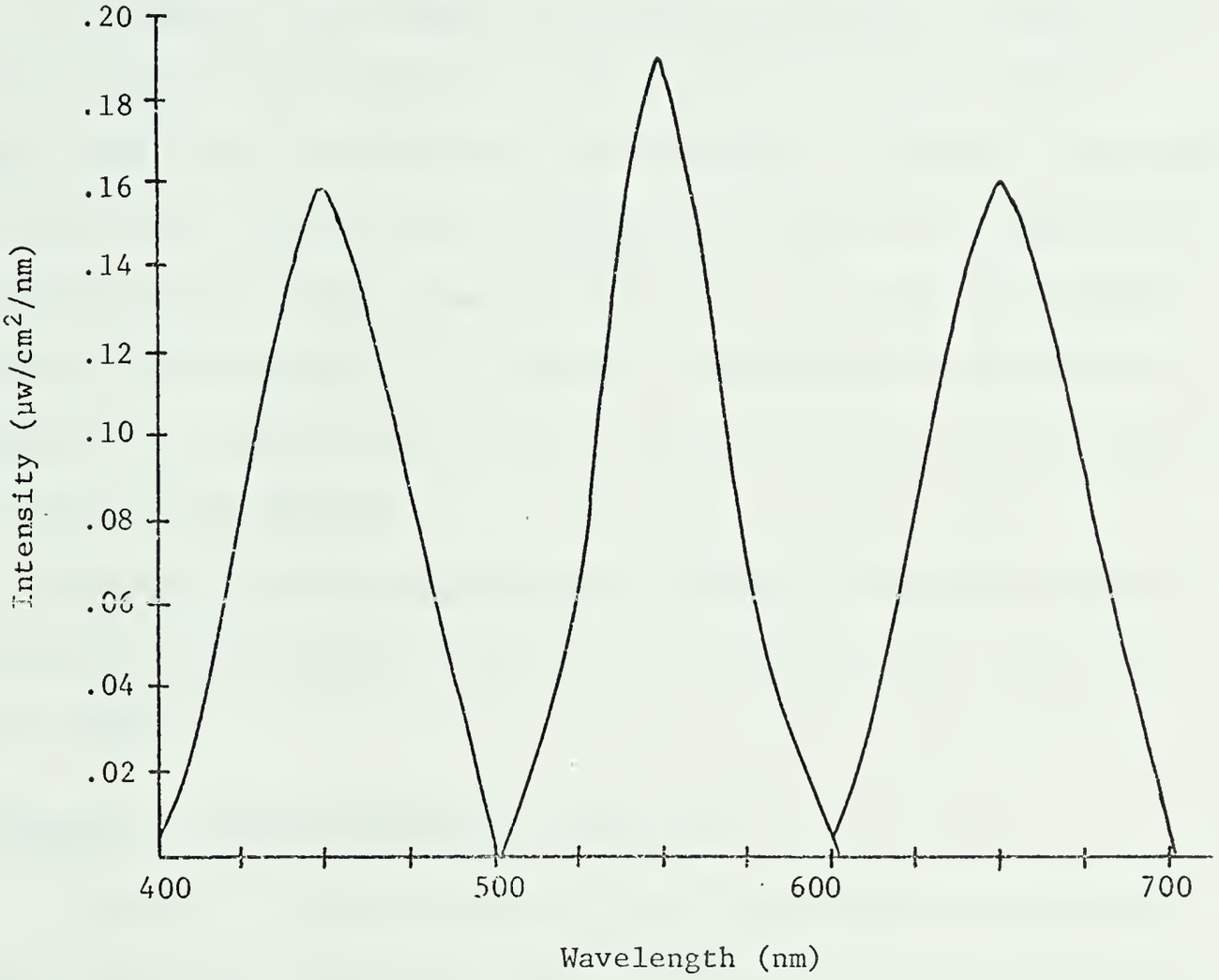

Figure 3. Spectral composition of light transmitted by CBS* filters and $CuSO_4$ in action spectrum experiment. The area under the energy transmittance curves was adjusted to be equal.

*Carolina Biological Supply Co., Burlington, North Carolina.

diluted to 1 cc with Spier and Meyer's fluid (Spier and Meyer, 1949). Eosinophils were counted in a Levy-Hausser counting chamber.* To avoid possible effects of stress on eosinophil levels, counts were taken on blood from undisturbed animals.

At 60 days of age animals were weighed and killed by holding a pencil behind the head and giving a quick pull on the tail. Fresh weights were taken for both testes, both adrenals and spleen. Pituitaries and ovaries were fixed in Bouin's solution and weighed on a Roller-Smith precision balance** after fixation for 24 hours. Ovaries were embedded in paraffin and sectioned at 10 microns. Representative sections (6 sections at 100 micron intervals) were stained and examined microscopically for follicular development.

The data from this experiment were analyzed by one-way analysis of variance and means compared by Duncan's multiple range test (Steel and Torrie, 1960).

Experiment 2. Stress eosinopenia in deer mice

To study the effect of handling and loss of blood on eosinophil levels, consecutive counts were taken of 8 female deer mice at ½ hour, 1 hour and 4 hours after an initial count.

Experiment 3. Influence of number of quanta of light on organ weights

Results of Experiment 1 suggested that changes in organ weights were possibly related to the number of quanta of light rather than to

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*Levy-Hausser corpuscle counting chamber, C. A. Hausser and Son, Philadelphia, Pa.

**Federal Pacific Electric Co., Newark, New Jersey.

energy level or wavelength *per se*. Thus in Experiment 3 four litters were reared in a blue environment and four litters were reared in a red environment with the number of quanta adjusted to be equivalent at peak filter transmittance in the two spectral environments. Energy intensities for these environments are shown in Figure 4. Since quantum energy is a physical constant, the number of quanta at the peak wavelength could be calculated according to the formula:

$$N = I\lambda/hc$$

where I is the radiant energy at a particular wavelength, λ, h is Planck's constant and c is the speed of light.

An additional four litters were reared in a blue environment with the number of quanta at peak filter transmittance adjusted to be equivalent to that calculated for the red environment of Experiment 1.

Organ weights were taken at 60 days of age as in Experiment 1.

Experiment 4. Influence of number of animals on organ weight

Because crowding has been reported to affect organ weights of mice (Bronson and Eleftheriou, 1963) two large litters (total 10 to 12 animals) from a dim white light environment, as described for Experiment 1, were reared together from weaning to 60 days of age, and a similar group in a red light environment. Organ weights were recorded at 60 days of age.

Experiment 5. Location of the photoreceptor

The eyes of mice from three litters of deer mice were enucleated at 12 to 14 days of age. The animals were reared from birth to 60 days of age under bright white light, as described for Experiment 1. Organ

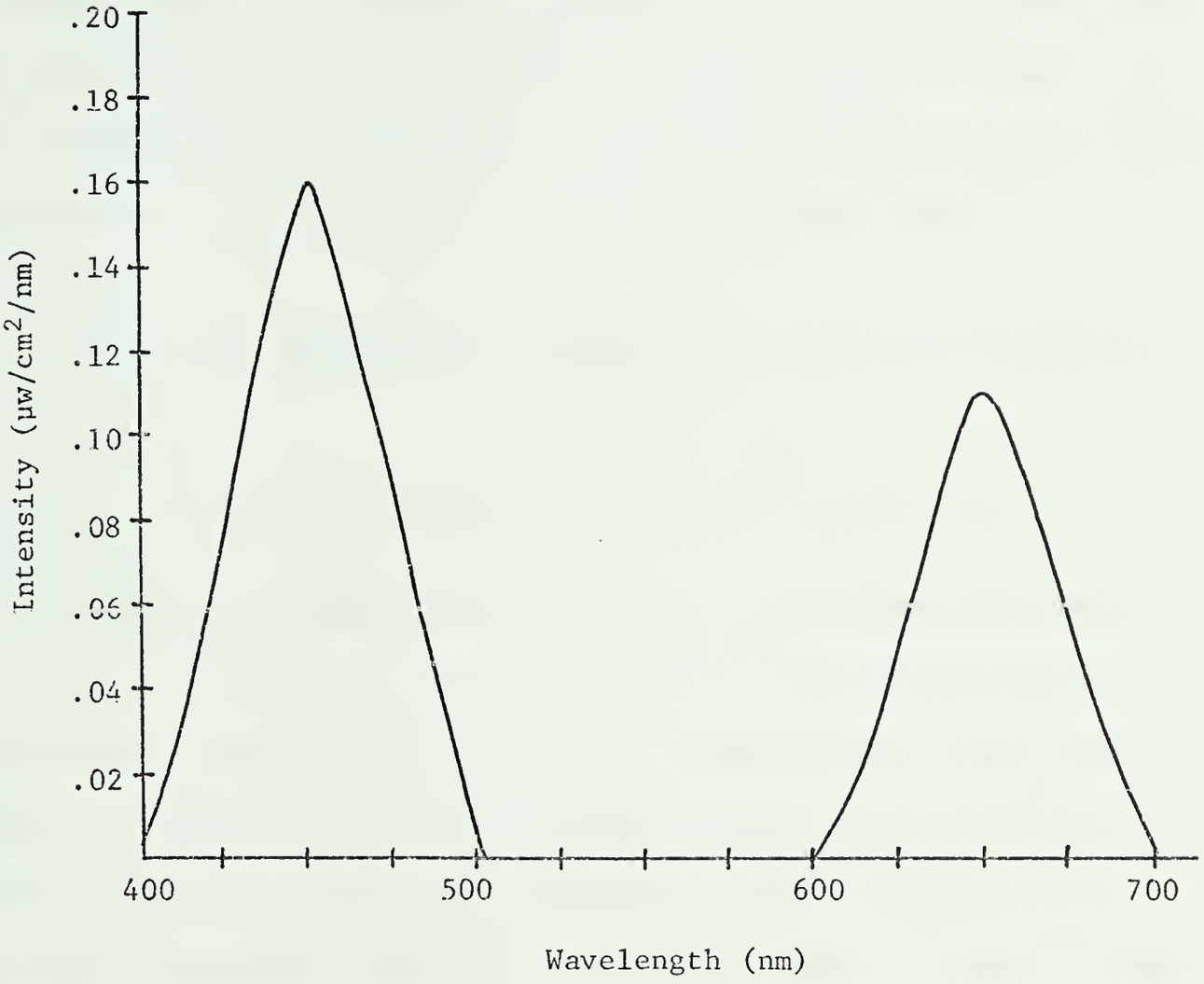

Figure 4. Spectral composition of light transmitted by coloured filters calibrated to pass equal number of quanta at peak wavelength. Experiment 3.

weights were recorded at 60 days of age.

Experiment 6. Influence of sex on adrenal weights

In an experiment designed to determine whether the effect of light on adrenal weight is influenced by gonadal factors, four litters of deer mice were reared in each of two white light environments, "bright" and "dim" as described for Experiment 1. Deer mice of both sexes were either gonadectomized or sham-operated at 21 days of age and maintained to 60 days of age.

The data from Experiments 3 through 6 were analyzed by Student's T test.

Experiment 7. Photoresponsiveness of mice from two inbred strains

Ten litters of BALB/c and 12 litters of C57/Bl mice were reared from birth in environments of several different colours and intensities. Litters were reduced at 14 days of age to 5 mice and the young were weaned at 21 days. Two of the environments were constant darkness (0L/24D). The other environments involved a 12L/12D photoperiod, and intensities and spectral qualities of 24 $\mu w/cm^2$ (red), 28 $\mu w/cm^2$ (white), 58 $\mu w/cm^2$ (white) and 94 $\mu w/cm^2$ (blue). Measured spectral intensities for these environments are shown in Figures 5 and 6.

From 58 to 60 days of age the mice were subjected to several emotionality tests. Data from this study are presented elsewhere.* When the mice had reached the age of 60 days this experiment was terminated and body weights and several organ weights were recorded. Data from 100 mice were analyzed by analysis of variance and means were compared by

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*Leo Mos, Department of Psychology, University of Alberta.

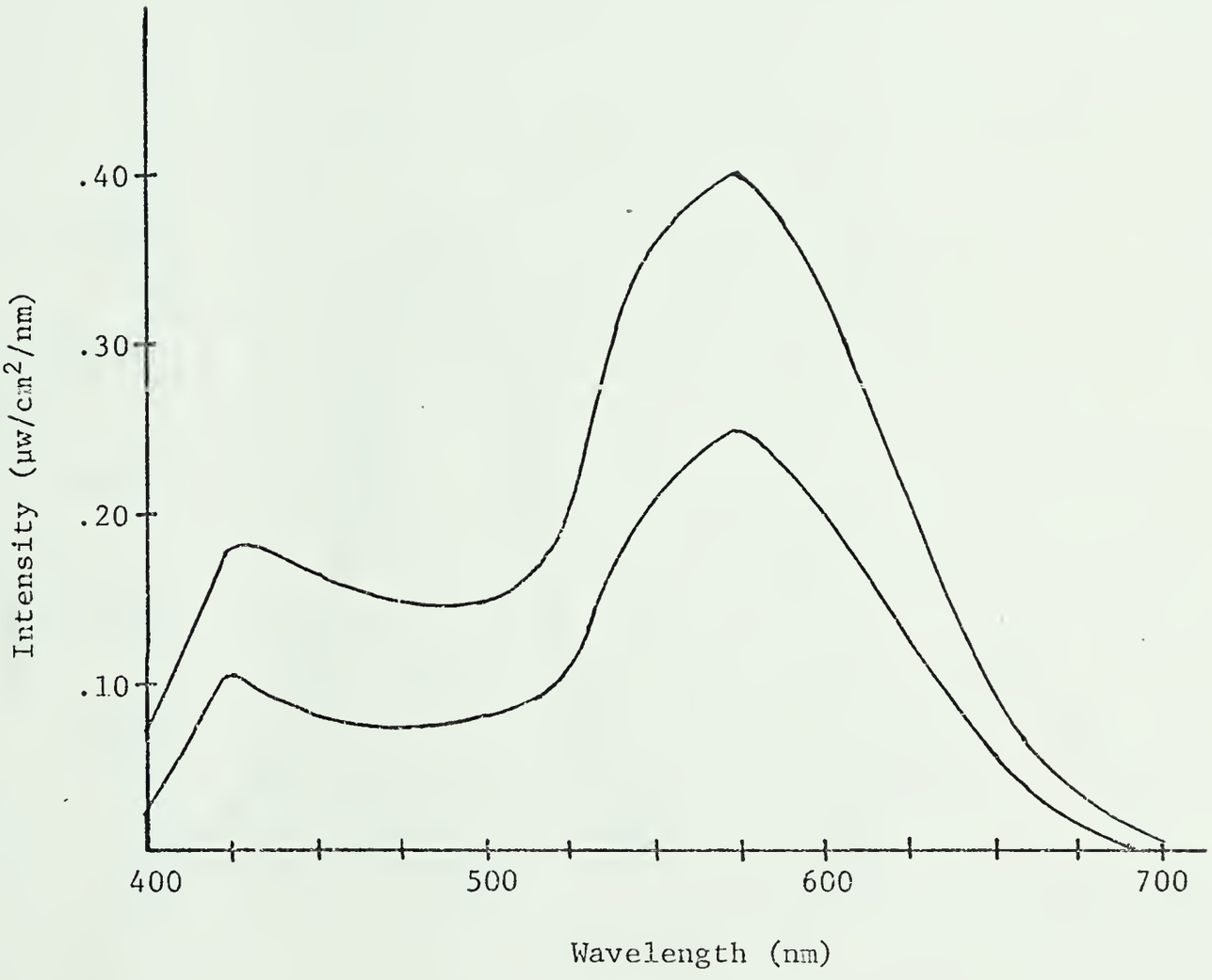


Figure 5. Spectral composition of fluorescent white light* used in Experiment 7.

*General Electric F40 CW.

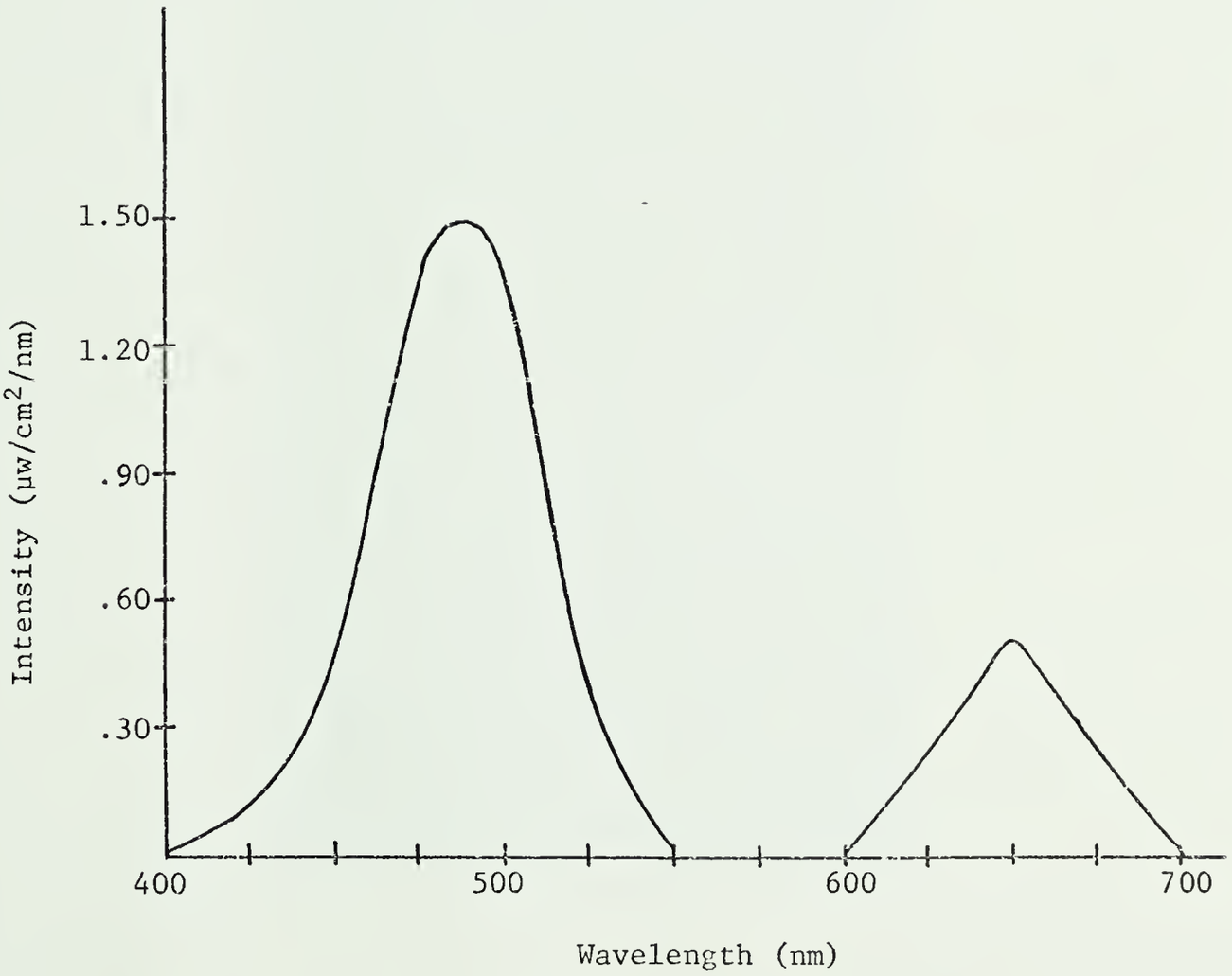


Figure 6. Spectral composition of light transmitted by coloured filters and $CuSO_4$ in Experiment 7.

Duncan's multiple range test (Steel and Torrie, 1960). Data from an additional ten C57/Bl mice in a second dark environment were a replication to serve as an added index of reliability. These data are presented in the Appendix.

Figure 7. Environment chamber

L - lamp
T - time clock
C - cupric sulphate
F - position of filter
t - thermometer

RESULTS

Studies on *Peromyscus maniculatus*

Experiment 1. Influence of light intensity and wavelength on organ weights and eosinophil counts

Body weight of deer mice increased with the intensity of light under which the litters were reared (Table I). Because of these body weight differences, gonad, adrenal and spleen weights are expressed relative to body weight rather than as absolute values.

Since the spectral environments elicited no differences in body weight or ovary, adrenal and pituitary weights, or in eosinophil counts, data from the three coloured light environments were combined. There were differences in these parameters when the combined data for the coloured light group were compared to the bright light group but these differences could be attributed to intensity (8.40 vs 2250 $\mu w/cm^2$).

The influence of wavelength and intensity on testis weight is shown in Table II and summarized graphically in Figure 8. Relative testis weight of deer mice varied considerably, from 1.16 to 8.19 mg/gm, between light treatments. Testis weight was greatest in the light environment of highest intensity (2250 $\mu w/cm^2$). In spectral environments of equal energy the response was greatest in red light, intermediate in green, and least in blue light and in darkness.

Relative ovary weight was greatest in animals reared under bright light (Table III and Figure 9) and significantly higher in both dim

Table I

Effect of light intensity on body weight of deer mice
(Experiment 1)

Light environment	Energy $\mu w/cm^2$	N	Sex	Body weight ± S.E.
Dark		28	F	14.05 ± .23
		23	M	15.71 ± .42
Coloured*	8.40	63	F	15.08 ± .20
		70	M	16.71 ± .23
Blue		14	F	14.90 ± .52
		20	M	16.20 ± .46
Green		19	F	15.59 ± .28
		28	M	17.01 ± .35
Red		30	F	14.78 ± .20
		22	M	16.81 ± .40
White (Dim)	16.00	13	F	15.68 ± .52
		17	M	17.85 ± .54
White (Bright)	2250.00	20	F	16.32 ± .40
		18	M	18.18 ± .54

Comparison of means

F	14.05	15.08	15.68	16.32	M	15.71	16.71	17.85	18.18
14.05	-	<.01	<.01	<.001	15.71	-	<.05	<.01	<.01
15.08		-	NS	<.01	16.71		-	<.05	<.01
15.68			-	NS	17.85			-	NS
16.32				-	18.18				-

F value NS for coloured groups.

*Total of three spectral environments. These data represent combined results for red, green and blue (see text) and may be viewed as a dim light group.

Table II

Testis weight of deer mice under several light intensities and several colours equated as to energy (Experiment 1)

Light environment	Energy $\mu w/cm^2$	N	Relative testis* weight mgTW/gmBW ± S.E.
Dark		23	1.16 ± .19
Blue	8.40	20	1.84 ± .38
Green	8.40	28	2.49 ± .39
Red	8.40	22	3.96 ± .92
White (Dim)	16.00	17	4.25 ± .68
White (Bright)	2250.00	18	8.19 ± 1.52

Comparison of means

	1.16	1.84	2.48	3.96	4.25	8.19
1.16	-	NS	<.10	<.01	<.01	<.001
1.84		-	NS	<.05	<.01	<.001
2.48			-	NS	<.10	<.001
3.96				-	NS	<.001
4.25					-	<.001
8.19						-

*Both testes.

Table III

Effect of light intensity on ovary weight of deer mice
(Experiment 1)

Light environment	Energy $\mu w/cm^2$	N	Relative ovary weight mgOW/gmBW ± S.E.
Dark		28	6.62 ± .48
Coloured*	8.40	63	12.36 ± 1.47
Blue		14	11.50 ± 1.30
Green		19	15.20 ± 4.00
Red		30	11.00 ± 1.70
White (Dim)	16.00	13	17.81 ± 3.92
White (Bright)	2250.00	20	21.99 ± 3.64

Comparison of means

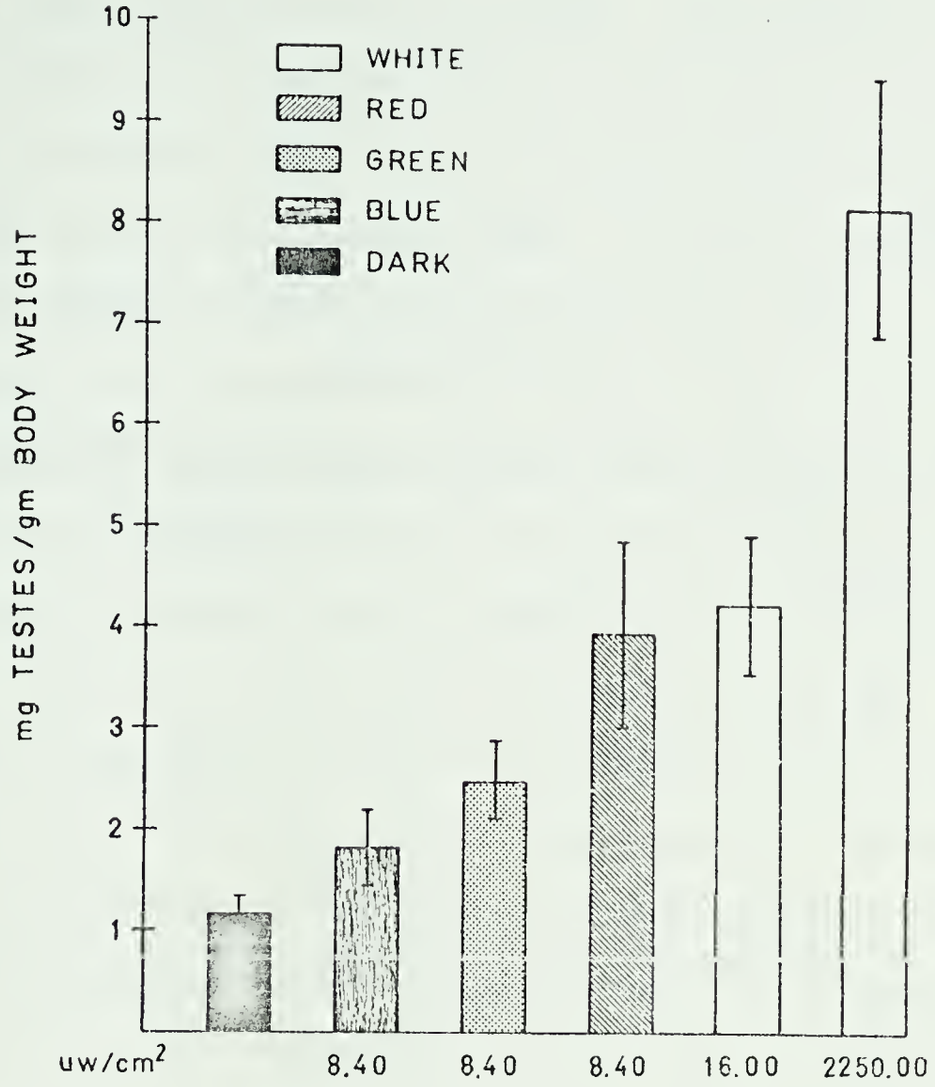
	6.62	12.36	17.81	21.99
6.62	-	<.05	<.01	<.001
12.36		-	NS	<.01
17.81			-	NS
21.99				-

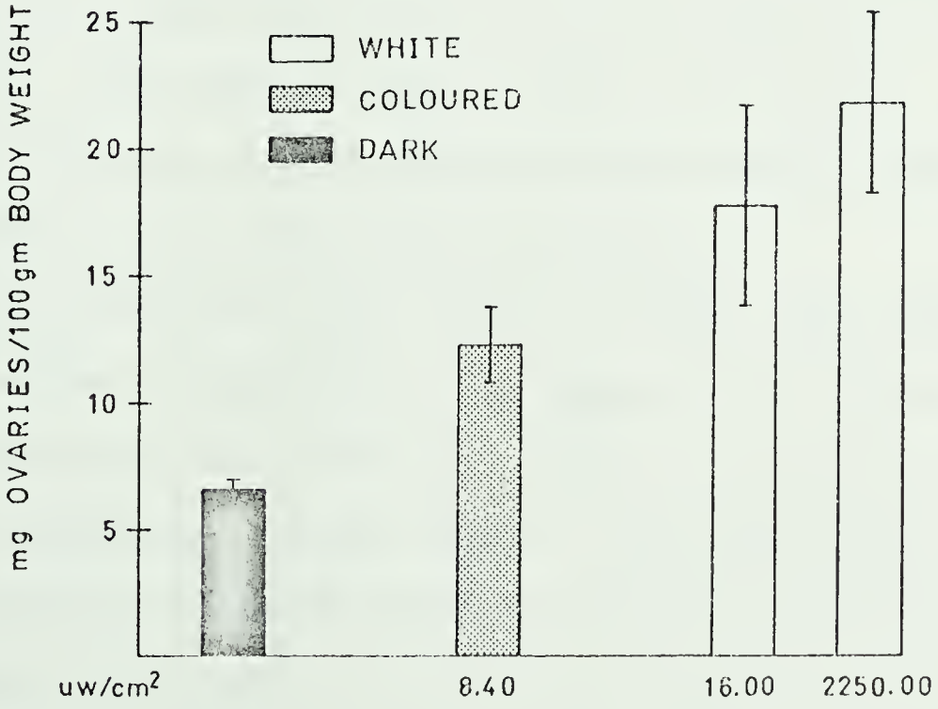
F value NS for coloured groups.

*Total of three spectral environments. These data represent combined results for red, green and blue (see text) and may be viewed as a dim light group.

Figure 8. Testes weight of deer mice under several light intensities and several colours equated as to energy. Age - 60 days. In this and following graphs vertical bars represent one standard error.

Figure 9. Effect of light intensity on ovary weight of deer mice. Age - 60 days. The coloured light bar represents combined data for red, green and blue environments (8.40 $\mu w/cm^2$) and is viewed as a dim light group.


mg TESTES/gm BODY WEIGHT
10
9
8
7
6
5
4
3
2
1
WHITE
RED
GREEN
BLUE
DARK
uw/cm²
8.40
8.40
8.40
16.00
2250.00


mg OVARIES/100gm BODY WEIGHT
25
20
15
10
5
WHITE
COLOURED
DARK
uw/cm²
8.40
16.00
2250.00

and bright light treatments than in the dark. Histological examination revealed primary and maturing follicles in the ovaries of mice reared in darkness and in dim light. Only in the ovaries of mice reared under bright light could corpora lutea be found. There was no difference in ovary weight between deer mice reared in the three spectral environments.

Of four litters reared in each of several light environments, 5 females from the bright light environment had a perforate vagina at 60 days, as well as two females from the dim white environment, one from the green and one from the blue environment; no females with perforate vagina at 60 days were found in the red or dark environment.

Table IV and Figure 10 show relative adrenal weights of male and female deer mice reared under several light conditions. Adrenal weight varied from 3.00 mg to 11.0 mg. Relative adrenal weight of female deer mice reared in dim white light was greater than that of females reared in darkness ($p < .01$). A similar trend toward greater adrenal weight was observed in males although this was not statistically significant. Male deer mice in bright light had lower adrenal weights than males in dim light ($p < .05$); a similar trend was observed in females. No significant difference could be detected between adrenal weights of deer mice reared in the three spectral environments.

Deer mice, like C57/Br mice (Spiers and Meyer, 1949), showed a change in peripheral eosinophil count in response to the stressful stimulus of handling and loss of blood. This was detectable after about an hour as a significant decrease (Exp. 2). Four hours after the stressful stimulus eosinophils had almost completely disappeared from the peripheral circulation.

In undisturbed animals eosinophils/mm^3 of blood were greater in animals reared in bright white light (2250 $\mu w/cm^2$) than in dim coloured

Table IV

Effect of light intensity on adrenal weight
of deer mice
(Experiment 1)

Light environment	Energy $\mu w/cm^2$	N	Sex	Relative adrenal weight mgAW/gmBW ± S.E.
Dark		28	F	.404 ± .017
		23	M	.374 ± .017
Coloured*	8.40	64	F	.441 ± .013
		70	M	.424 ± .012
Blue		14	F	.473 ± .024
		20	M	.444 ± .020
Green		20	F	.446 ± .025
		28	M	.406 ± .020
Red		30	F	.423 ± .022
		22	M	.437 ± .028
White (Dim)	16.00	13	F	.504 ± .039
		17	M	.423 ± .031
White (Bright)	2250.00	20	F	.451 ± .023
		18	M	.358 ± .017

Comparison of means

F	.404	.441	.451	.504	M	.358	.374	.423	.424
.404	-	NS	NS	<.01	.358	-	NS	NS	<.05
.441		-	NS	NS	.374		-	NS	NS
.451			-	NS	.423			-	NS
.504				-	.424				-

F value NS for coloured groups.

*Total of three spectral environments. These data represent combined results for red, green and blue (see text) and may be viewed as a dim light group.

Figure 10. Effect of light intensity on adrenal weight of deer mice. Age - 60 days. The coloured light bar represents combined data for red, green and blue environments (8.40 $\mu w/cm^2$) and is viewed as a dim light group.

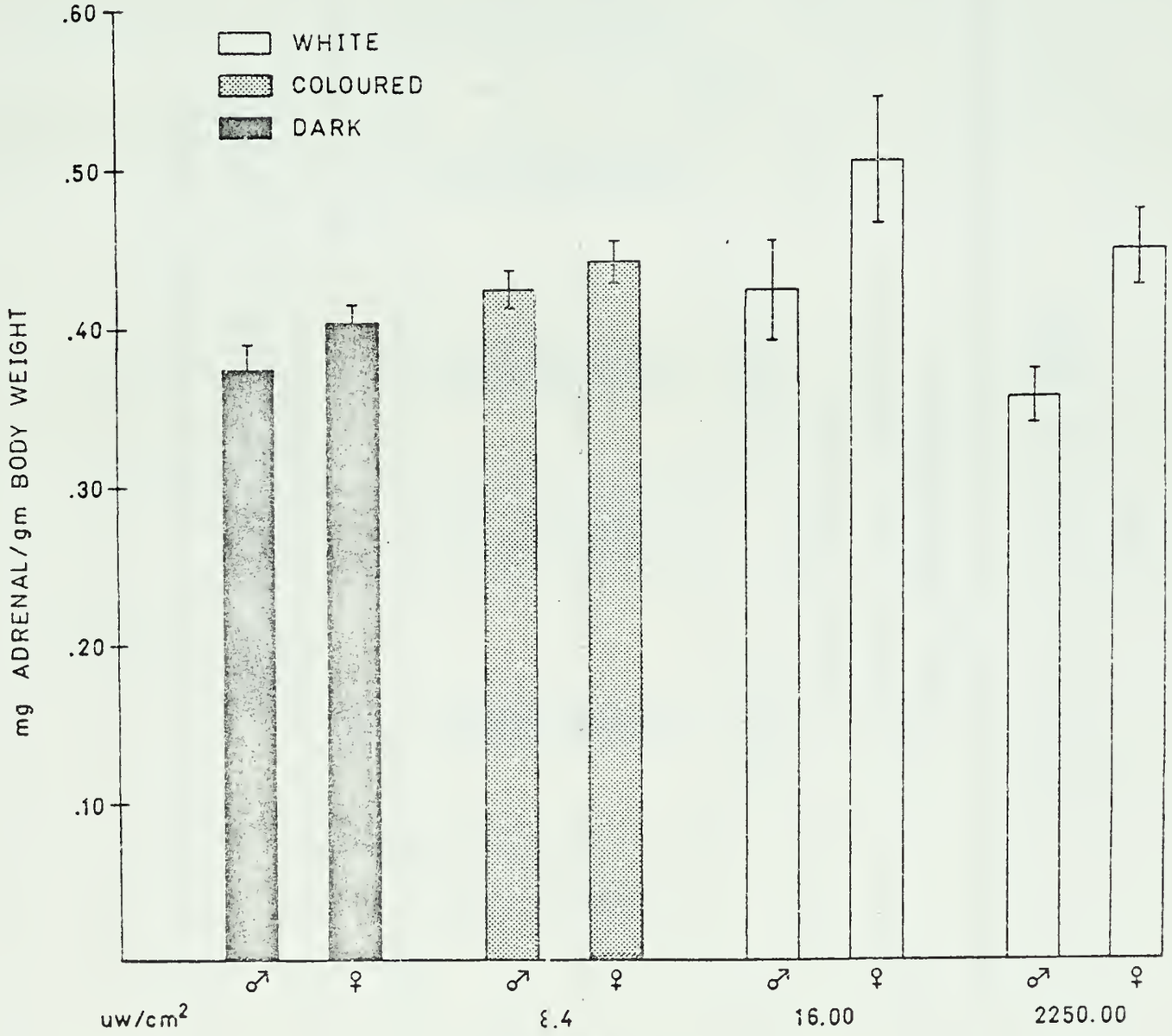

.60
.50
.40
.30
.20
.10
WHITE
COLOURED
DARK
mg ADRENAL/gm BODY WEIGHT
♂
♀
uw/cm²
8.4
16.00
2250.00

Table V

Stress eosinopenia* in deer mice

Eosinophils/mm^3 blood					
Time after handling					
0 hr	1/2 hr	% change**	1 hr	% change**	4 hr	% change**
500	588	+18	267	-46	55	-89
355	422	+19	177	-50	22	-94
1278	955	-25	577	-55	308	-76
167	222	+33	111	-33	11	-93
355	319	-10	244	-31	33	-91
222	233	+ 5	88	-60	11	-95
127	111	-13	66	-48	0	-100
400	466	+17	222	-45	66	-84
Average % change		+ 5.5		-46		-90

*Induced by handling and loss of blood.

**Percent change as compared with count on same animal at 0 hr.

Table VI

Effect of light intensity on circulating eosinophils of deer mice (Experiment 1)

Light environment	Energy $\mu w/cm^2$	N	Sex	Eosinophils/mm^3 tail blood ± S.E.
Dark		17	F	157 ± 32
		12	M	118 ± 23
Coloured*	8.40	46	F	271 ± 26
		45	M	307 ± 24
Blue		18	F	337 ± 51
		12	M	307 ± 59
Green		14	F	244 ± 34
		20	M	302 ± 36
Red		14	F	212 ± 34
		12	M	335 ± 39
White (Dim)	16.00	4	F	263 ± 57
		9	M	317 ± 69
White (White)	2250.00	13	F	384 ± 76
		12	M	409 ± 68

Comparison of means

F	157	263	271	384	M	118	307	317	409
157	-	NS	<.05	<.01	118	-	<.01	<.05	<.001
263		-	NS	NS	307		-	NS	NS
271			-	<.05	317			-	NS
384				-	384				-

F value for coloured groups NS.

*Total of three coloured environments. These data represent combined results for red, green and blue (see text) and may be viewed as a dim group.

Figure 11. Effect of light intensity on circulating eosinophils of deer mice. Age - 42 days. The coloured light bar represents combined data for red, green and blue environments (8.40 $\mu w/cm^2$) and is viewed as a dim light group.

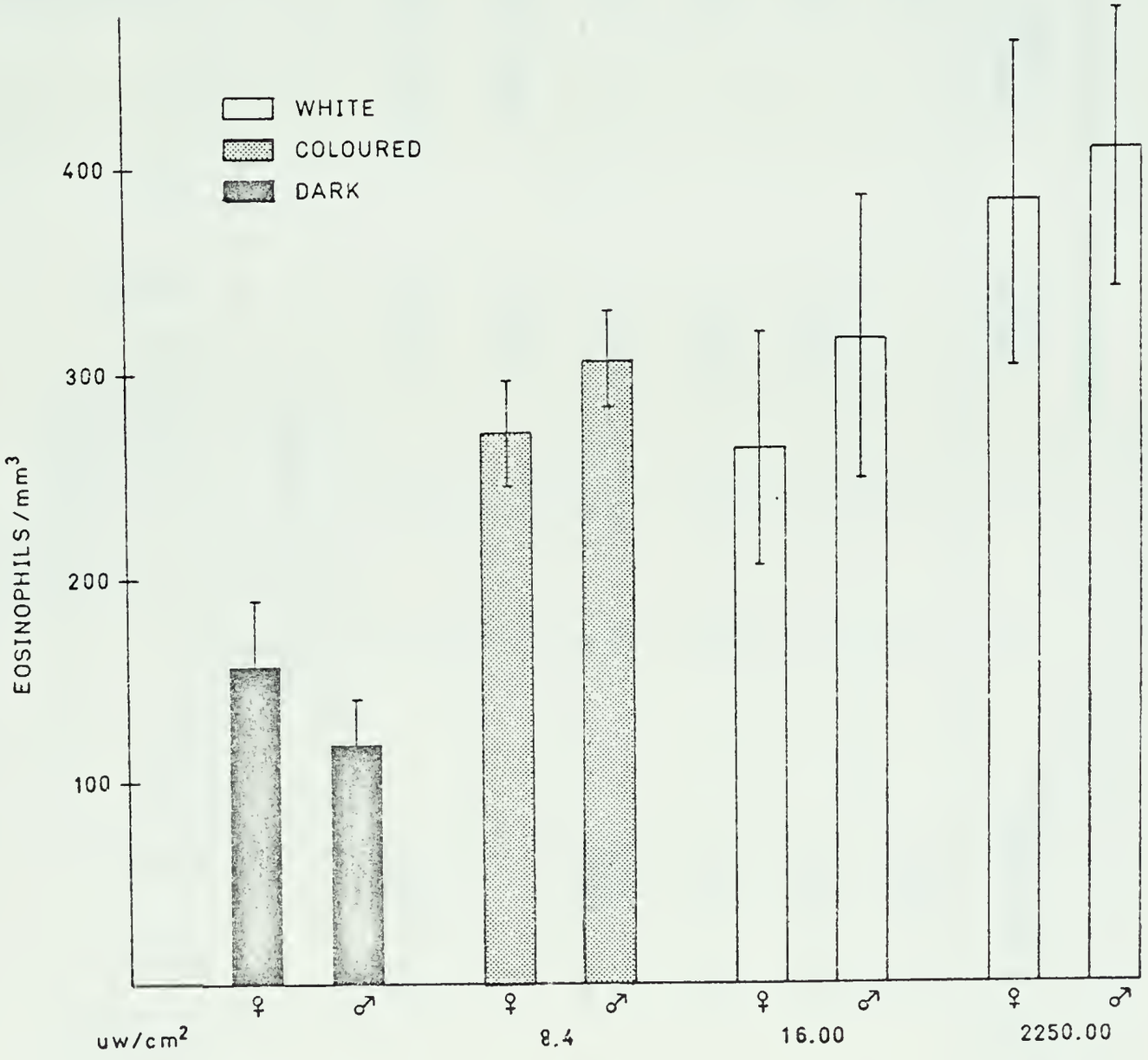

WHITE
COLOURED
DARK
400
300
200
100
EOSINOPHILS / mm³
♀
♂
♀
♂
♀
♂
♀
♂
uw/cm²
8.4
16.00
2250.00

Table VII

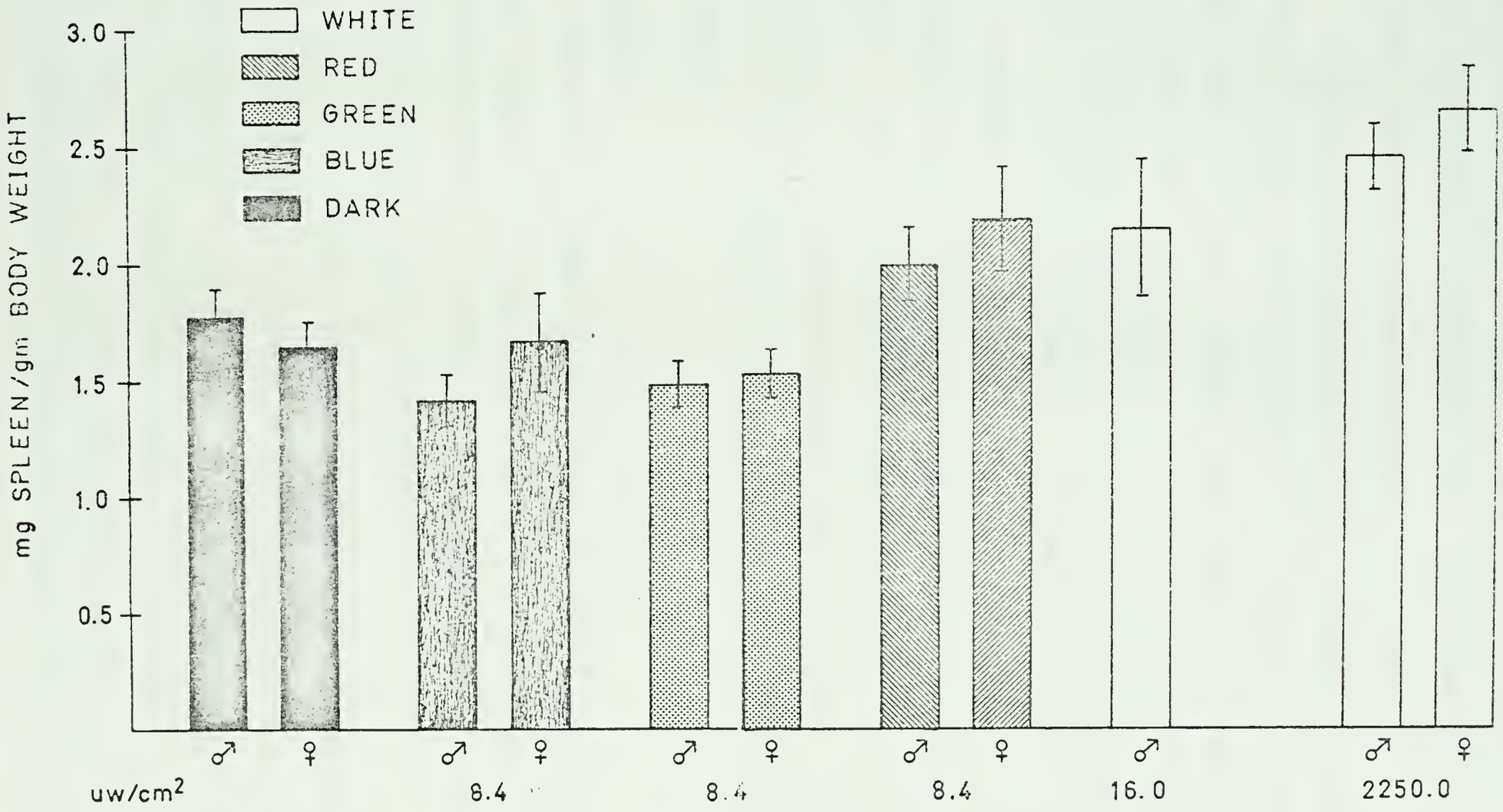
Spleen weight of deer mice under several light intensities and several colours equated as to energy (Experiment 1)

Light environment	Energy $\mu w/cm^2$	N	Sex	Relative spleen weight mgSW/gmBW ± S.E.
Dark		17	F	1.65 ± .11
		15	M	1.78 ± .12
Blue	8.40	6	F	1.67 ± .21
		10	M	1.42 ± .11
Green	8.40	15	F	1.53 ± .10
		13	M	1.49 ± .10
Red	8.40	9	F	2.19 ± .22
		12	M	2.00 ± .16
White (Dim)	16.00	-	-	
		6	M	2.15 ± .29
White (Bright)	2250.00	12	F	2.65 ± .18
		10	M	2.45 ± .14

Comparison of means

F	1.65	1.67	1.53	2.19	2.65	M	1.42	1.49	1.78	2.00	2.15	2.45
1.65	-	NS	NS	<.01	<.001	1.42	-	NS	NS	<.05	<.01	<.001
1.67		-	NS	<.01	<.001	1.49		-	NS	<.05	<.05	<.001
1.53			-	NS	<.001	1.78			-	NS	NS	<.01
2.19				-	<.05	2.00				-	NS	<.05
2.65					-	2.15					-	NS
						2.45						-

Figure 12. Spleen weight of deer mice under several light intensities and several colours equated as to energy. Age - 60 days.


WHITE
RED
GREEN
BLUE
DARK
mg SPLEEN/gm BODY WEIGHT
3.0
2.5
2.0
1.5
1.0
0.5
♂
♀
uw/cm²
8.4
8.4
8.4
16.0
2250.0

light (8.40 $\mu w/cm^2$) or in darkness (Table VI and Figure 11). There were no differences in eosinophil counts between deer mice reared under different spectral environments equated as to energy.

Table VII and Figure 12 summarize the influence of wavelength and intensity on spleen weight relative to body weight. Spleen weight varied from 15 to 70 mg. Relative spleen weight of animals reared in dim or bright white light (16 or 2250 $\mu w/cm^2$) or in red light (8.4 $\mu w/cm^2$) was greater than that of animals reared in blue light or green light (8.4 $\mu w/cm^2$) or in darkness. This was true for both sexes.

Pituitary weight, shown in Table VIII and Figure 13, was lowest in animals reared in darkness and highest in animals reared in bright light ($p < .001$). Intermediate weights were recorded for animals reared in white light of intensity 16 $\mu w/cm^2$ and coloured light of intensity 8.4 $\mu w/cm^2$. No differences in pituitary weight could be attributed to wavelengths.

Experiment 2. Stress eosinopenia in deer mice

The results, shown in Table V, confirm that handling and blood loss cause eosinopenia, but this effect is first evident one hour after stress. This substantiates the validity of light effects on eosinophil counts of undisturbed animals, shown in Experiment 1.

Experiment 3. Influence of number of quanta on organ weights of deer mice

The differential response of testis and spleen to wavelength of light demonstrated in Experiment 1 was not observed when the number of quanta at peak wavelength were adjusted to be equivalent in blue and red environments. This was so when the number of quanta at peak was 3.64×10^{11} or when the number of quanta at peak was increased to 5.60×10^{11}.

Table VIII

Effect of light intensity on pituitary weight of deer mice
(Experiment 1)

Light environment	Energy $\mu w/cm^2$	N	Sex	Pituitary weight (absolute) mg ± S.E.
Dark		17	F	.46 ± .02
		15	M	.43 ± .03
Coloured*	8.40	32	F	.54 ± .02
		34	M	.54 ± .01
Blue		10	F	.57 ± .02
		7	M	.57 ± .02
Green		15	F	.56 ± .02
		13	M	.53 ± .02
Red		10	F	.50 ± .04
		11	M	.54 ± .02
White (Dim)	16.00	8	F	.57 ± .04
		8	M	.60 ± .04
White (Bright)	2250.00	12	F	.63 ± .02
		10	M	.62 ± .03

Comparison of means

F	.46	.54	.57	.63	M	.43	.54	.57	.62
.46	-	<.01	<.01	<.001	.43	-	<.001	<.001	<.001
.54		-	NS	<.05	.54		-	NS	<.05
.57			-	NS	.57			-	NS
.63				-	.62				-

F value NS for coloured groups.

*Total of three spectral environments. These data represent combined results for red, green and blue (see text) and may be viewed as a dim light group.

Figure 13. Effect of light intensity on pituitary weight of deer mice. Age - 60 days. The coloured light bar represents combined data for red, green and blue environments (8.40 $\mu w/cm^2$) and is viewed as a dim light group.

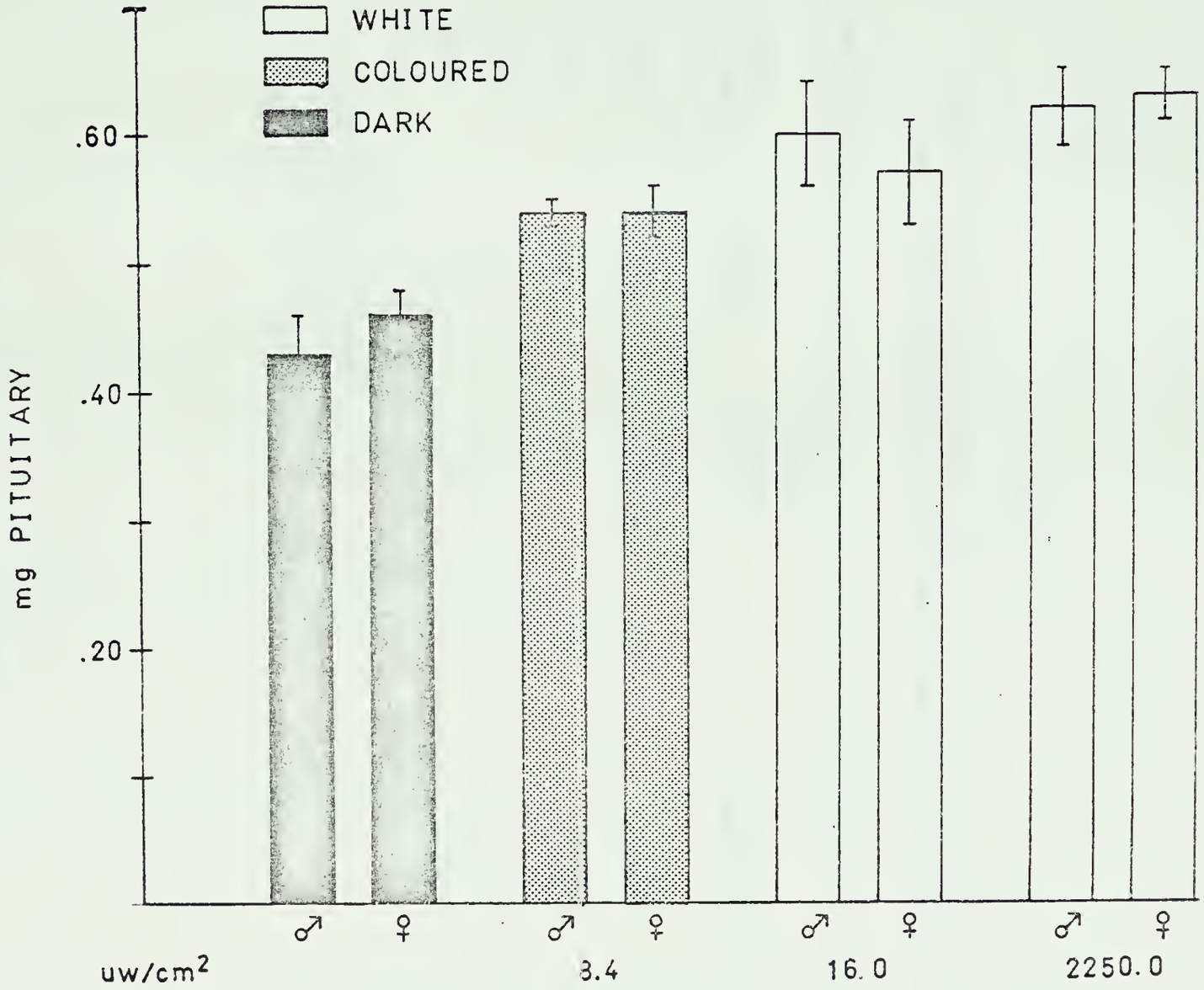

WHITE
COLOURED
DARK
.60
.40
.20
mg PITUITARY
♂
♀
♂
♀
♂
♀
♂
♀
uw/cm²
3.4
16.0
2250.0

Table IX

Testis weight of deer mice under several light intensities and several colours equated as to number of quanta

Light environment	Energy $\mu w/cm^2$	Quanta at peak λ x 10^{11}	N	Relative testis weight mgTW/gmBW ± S.E.
1 Blue	8.40	3.64	20	1.84 ± .38
2 Red	6.10	3.64	11	2.06 ± .84
3 Blue	9.20	5.60	13	3.37 ± 1.00
4 Red	8.40	5.60	22	3.96 ± .92

By Student's T Test
1:2 NS, 3:4 NS,
1+2:3+4, $p < .05$.

Figure 14. Testis weight of deer mice under several light intensities and several colours equated as to number of quanta. Age - 60 days.

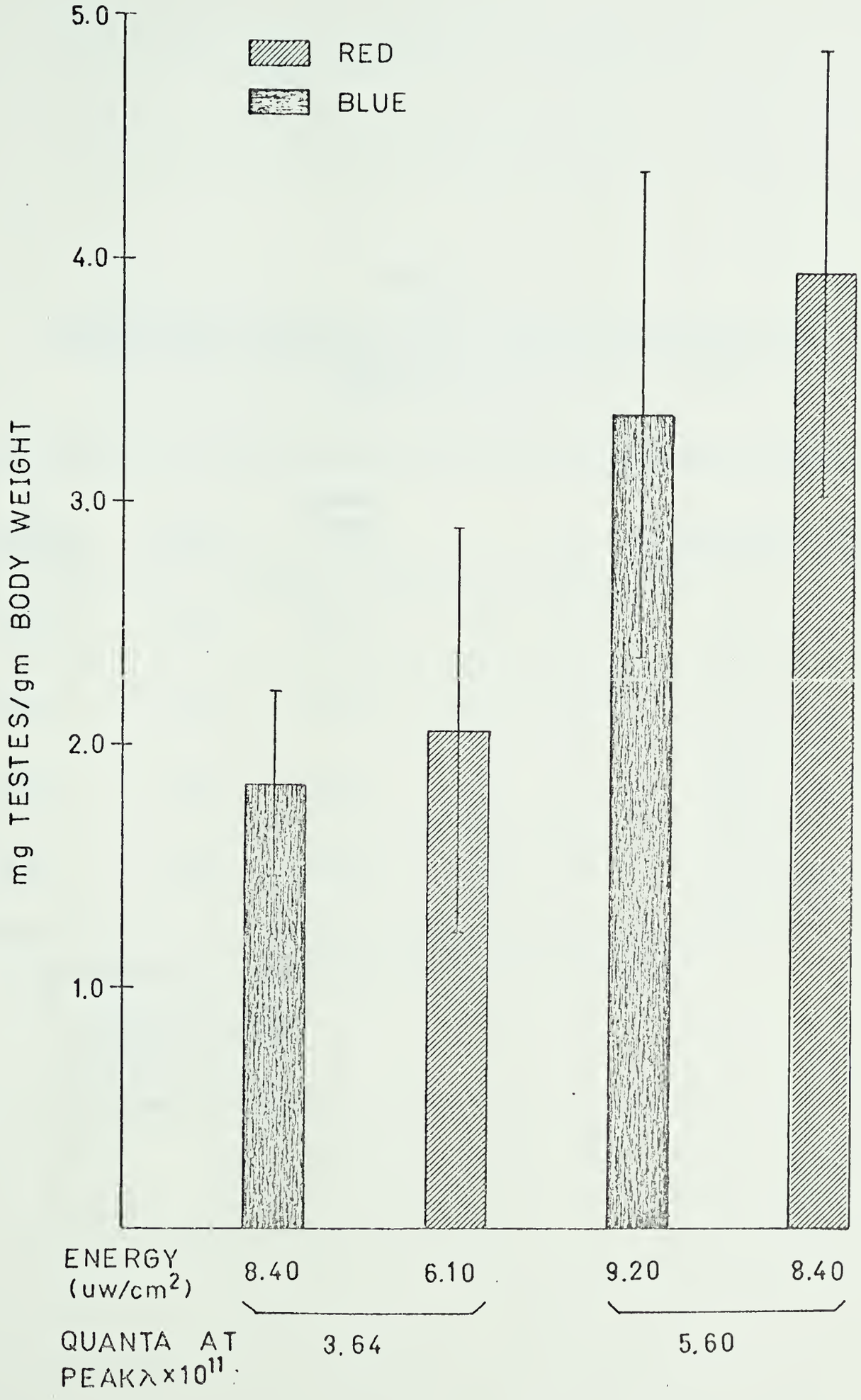

5.0
4.0
3.0
2.0
1.0
RED
BLUE
mg TESTES/gm BODY WEIGHT
ENERGY (uw/cm²)
8.40
6.10
9.20
8.40
QUANTA AT PEAKλ x10¹¹
3.64
5.60

Table X

Spleen weight of deer mice under several light intensities and several colours equated as to number of quanta (Experiment 3)

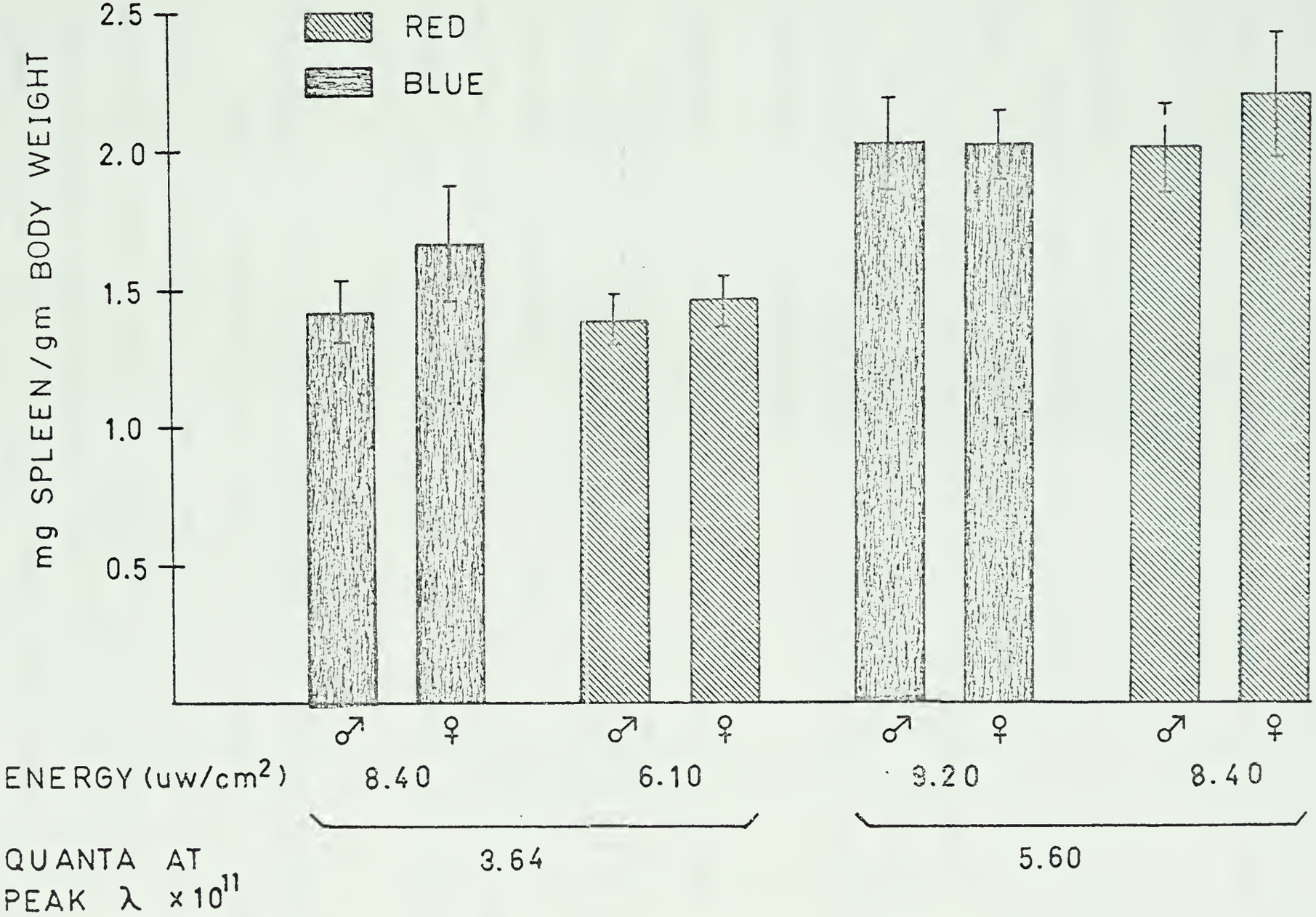
Light environment	Energy $\mu w/cm^2$	Quanta at peak λ x 10^{11}	N	Sex	Relative spleen weight mgSW/gmBW ± S.E.
1 Blue	8.40	3.64	6	F	1.67 ± .21
			10	M	1.42 ± .11
2 Red	6.10	3.64	10	F	1.46 ± .09
			11	M	1.39 ± .09
3 Blue	9.20	5.60	13	F	2.01 ± .12
			13	M	2.02 ± .17
4 Red	8.40	5.60	9	F	2.19 ± .22
			12	M	2.00 ± .16

By Student's T Test

♀ 1:2 NS, 3:4 NS, 1+2:3+4, $p < .01$.

♂ 1:2 NS, 3:4 NS, 1+2:3+4, $p < .01$.

Figure 15. Spleen weight of deer mice under several light intensities and several colours equated as to number of quanta. Age - 60 days.


2.5
2.0
1.5
1.0
0.5
mg SPLEEN/gm BODY WEIGHT
RED
BLUE
♂
♀
♂
♀
♂
♀
♂
♀
ENERGY (uw/cm²)
8.40
6.10
3.20
8.40
QUANTA AT PEAK λ x10¹¹
3.64
5.60

Testis weight (Table IX and Figure 14) and spleen weight of both sexes (Table X and Figure 15) were greater in environments with the greater number of quanta at peak wavelength, irrespective of colour.

Experiment 4. Influence of number of animals per cage on organ weights

When the number of male deer mice was increased after weaning to 10 per cage (as compared to an average of 5 in other experiments) testis, spleen and pituitary weights were lower than for uncrowded controls ($p < .05$) (see Table XI). The effect was somewhat less (see Table XI for significance) in white light (16 $\mu w/cm^2$) than in red light (8.4 $\mu w/cm^2$) with respect to all organs weighed.

Experiment 5. The eye as photoreceptor for photoendocrine responses

Table XII gives organ weights relative to body weight of deer mice blinded at 12 to 14 days of age and reared in bright white light as described for Experiment 1. Adrenal weights of enucleated mice of both sexes were lower than those for intact control animals (see Table IV). Relative ovary, testis and spleen weights were comparable to those of animals reared in darkness.

Experiment 6. Effect of light intensity and gonadectomy on organ weights

Table XIII summarizes the influence of light intensity on organ weights of castrated mice. Pituitary weight of spayed females was significantly greater than that of sham-gonadectomized animals or unoperated litter mate controls ($p < .05$). Male pituitary weights followed a similar trend. The weight of the thymus was greater in castrated males than in intact males reared in dim light ($p < .05$). There was no significant difference in thymus weight between castrated

Table XI

Influence of number of animals per cage on organ weights of male deer mice

		Adrenal mg/gmBW ± S.E.	Testis mg/gmBW ± S.E.	Spleen mg/gmBW ± S.E.	Pituitary mg ± S.E.	N
A.	Red	.344 ± .030	.69 ± .07	1.23 ± .08	.45 ± .02	10*
B.	Controls (Exp. 1)	.424 ± .012	3.96 ± .92	2.00 ± .16	.54 ± .01	
C.	White	.455 ± .022	1.13 ± .32	1.73 ± .12	.49 ± .02	10
D.	Controls (Exp. 1)	.423 ± .031	4.25 ± .68	2.15 ± .29	.60 ± .04	

By Student's T Test:
A:C $p < .05$ for adrenal and spleen weights
A:B $p < .05$ for adrenal, testis, spleen and pituitary weights
C:D $p < .05$ for testis and pituitary weights.

*As compared with an average litter size of 5 for controls.

Table XII

Organ weights of deer mice blinded at 12 to 14 days of age
(Experiment 5)

	Adrenal mg/gmBW ± S.E.	Gonad mg/gmBW ± S.E.	Spleen mg/gmBW ± S.E.	Pituitary mg ± S.E.	N
A. ♀ Blinded	.345 ± .020	.065 ± .006	1.76 ± .14	.50 ± .03	10
B. ♀ Intact (Exp. 1)	.451 ± .023	.220 ± .036	---	.63 ± .02	
C. ♂ Blinded	.310 ± .008	1.28 ± .60	1.71 ± .20	.55 ± .02	4
D. ♂ Intact (Exp. 1)	.358 ± .017	8.19 ± 1.52	2.15 ± .29	.62 ± .03	

By Student's T Test:
A:B $p < .01$ for adrenal and gonad; $p < .05$ for pituitary
C:D $p < .05$ for adrenal and spleen; $p < .01$ for gonad.

Table XIII

Effect of light intensity and gonadectomy
on organ weights of deer mice
(Experiment 6)

Dim light	N	Adrenal mg/gmBW ± S.E.	Spleen mg/gmBW ± S.E.	Thymus mg/gmBW ± S.E.	Pituitary mg ± S.E.
Spayed ♀	3	.346 ± .039	1.65 ± .31	2.30 ± .63	.86 ± .14
Sham operated ♀	4	.414 ± .027	1.32 ± .14	2.21 ± .47	.54 ± .11
Unoperated ♀	1	.465	1.55	2.35	.64
Castrated ♂	6	.362 ± .016	2.01 ± .23	3.04 ± .30	.65 ± .04
Sham operated ♂	4	.429 ± .031	1.77 ± .15	2.19 ± .20	.48 ± .04
Unoperated ♂	7	.393 ± .037	1.69 ± .18	1.99 ± .12	.62 ± .05
Bright light					
Spayed ♀	3	.450 ± .050	1.60 ± .11	2.32 ± .28	.92 ± .04
Sham operated ♀	3	.392 ± .032	1.82 ± .07	1.70 ± .38	.62 ± .04
Unoperated ♀	4	.379 ± .043	1.46 ± .10	2.13 ± .15	.52 ± .03
Castrated ♂	6	.384 ± .040	1.46 ± .18	2.25 ± .31	.62 ± .03
Sham operated ♂	4	.416 ± .053	1.53 ± .13	2.28 ± .25	.54 ± .05
Unoperated ♂	3	.349 ± .008	2.07 ± .09	1.98 ± .13	.57 ± .03

males and controls reared in bright light.

Studies on Inbred Strains

Experiment 7. Responses of inbred mice to several light treatments

Experiment 7 was designed to test the responses of male and female mice from two inbred strains of *Mus musculus* to five different lighting treatments (2 x 2 x 5 factorial design).

Figures 16 to 23 portray graphically the organ weight findings of Experiment 7. Included with the C57/Bl data are organ weights of 10 animals not included in the factorial design. These animals were reared in a second dark environment; data from these animals (Appendix B) serve as an added index of comparison.

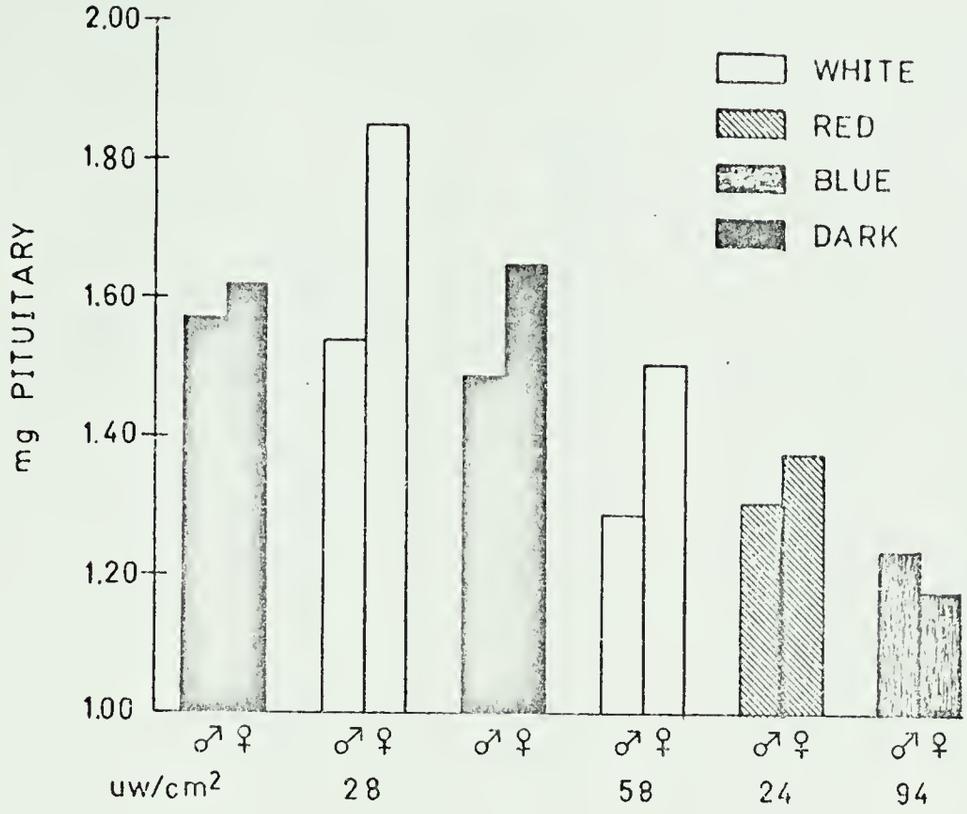
At 60 days of age both C57/Bl and BALB/c mice have reached adult body weights and are post-puberal animals. Since no significant difference in body weight was revealed by factorial analysis of variance (Appendix A), absolute organ weights were studied. The data on body weights of C57/Bl mice were not as replicable as were data on organ weights (see Figures 16 to 19) of these mice.

Figures 16 and 17 show organ weights of C57/Bl mice under five lighting treatments. Both pituitary and gonad weights of mice reared under white light (28 $\mu w/cm^2$) were higher than in darkness. Pituitary and gonad weights of animals reared under higher intensity white light (58 $\mu w/cm^2$), red light (24 $\mu w/cm^2$) or blue light (94 $\mu w/cm^2$) were lower than those of animals reared in darkness. (See Tables XIV B and XV B for comparison of means.)

Figures 20 and 21 similarly show organ weights for BALB/c mice.

Figure 16. Pituitary weight of C57/Bl mice under several light intensities. Age - 60 days.

Figure 17. Gonad weight of C57/Bl mice under several light intensities. Age - 60 days.


2.00
1.80
1.60
1.40
1.20
1.00
mg PITUITARY
WHITE
RED
BLUE
DARK
♂ ♀
uw/cm²
28
58
24
94

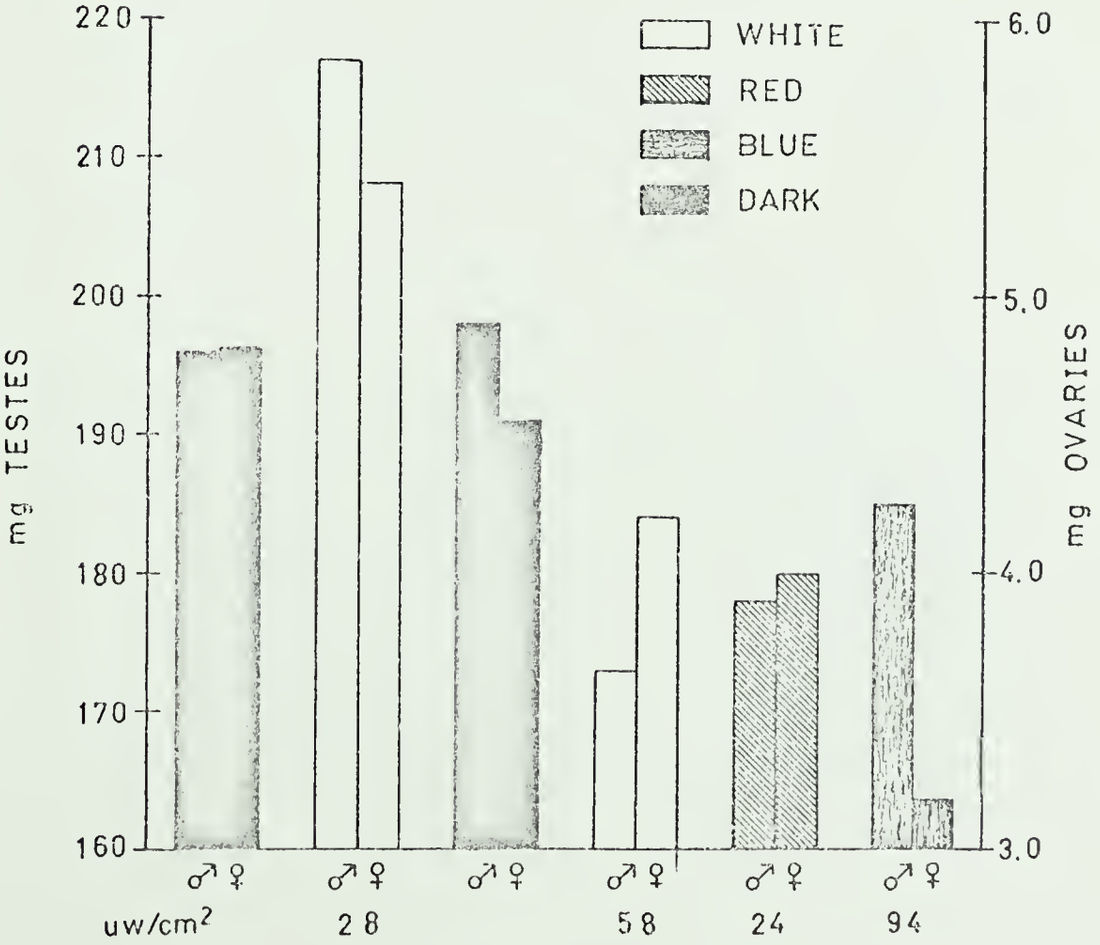
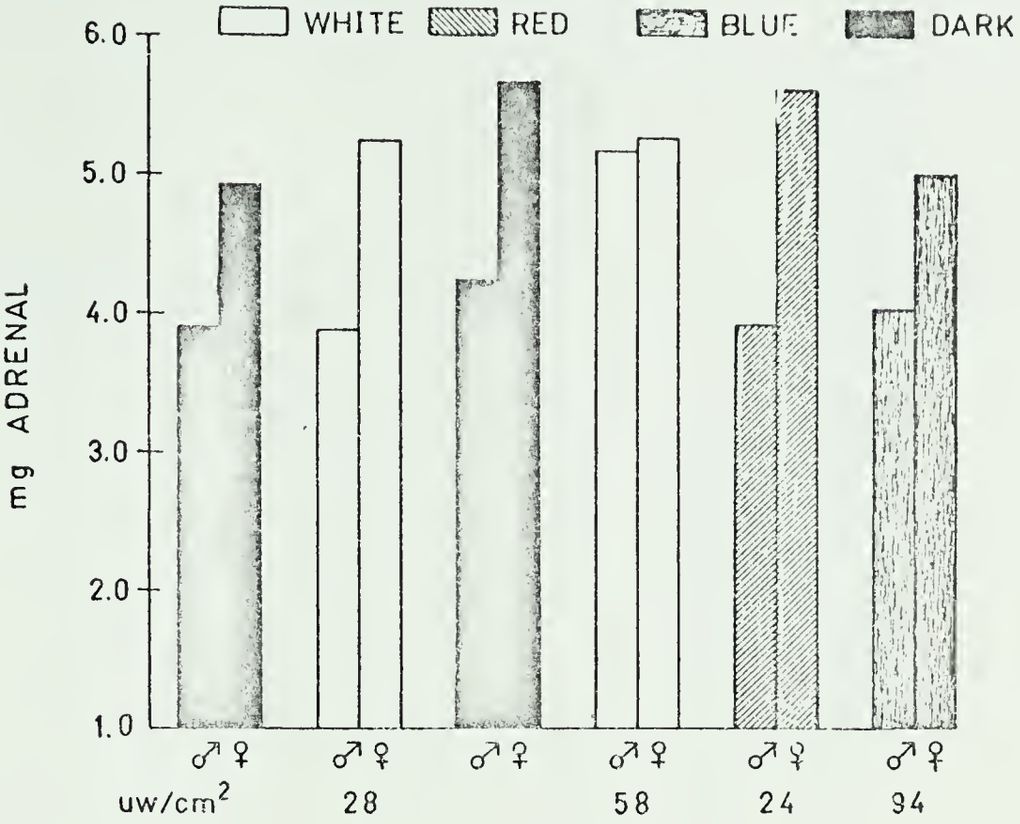

220
210
200
190
180
170
160
mg TESTES
6.0
5.0
4.0
3.0
mg OVARIES
WHITE
RED
BLUE
DARK
♂ ♀
uw/cm²
28
58
24
94

Figure 18. Adrenal weight of C57/Bl mice under several light intensities. Age - 60 days.

Figure 19. Spleen weight of C57/Bl mice under several light intensities. Age - 60 days.


WHITE
RED
BLUE
DARK
mg ADRENAL
6.0
5.0
4.0
3.0
2.0
1.0
♂ ♀
uw/cm²
28
58
24
94

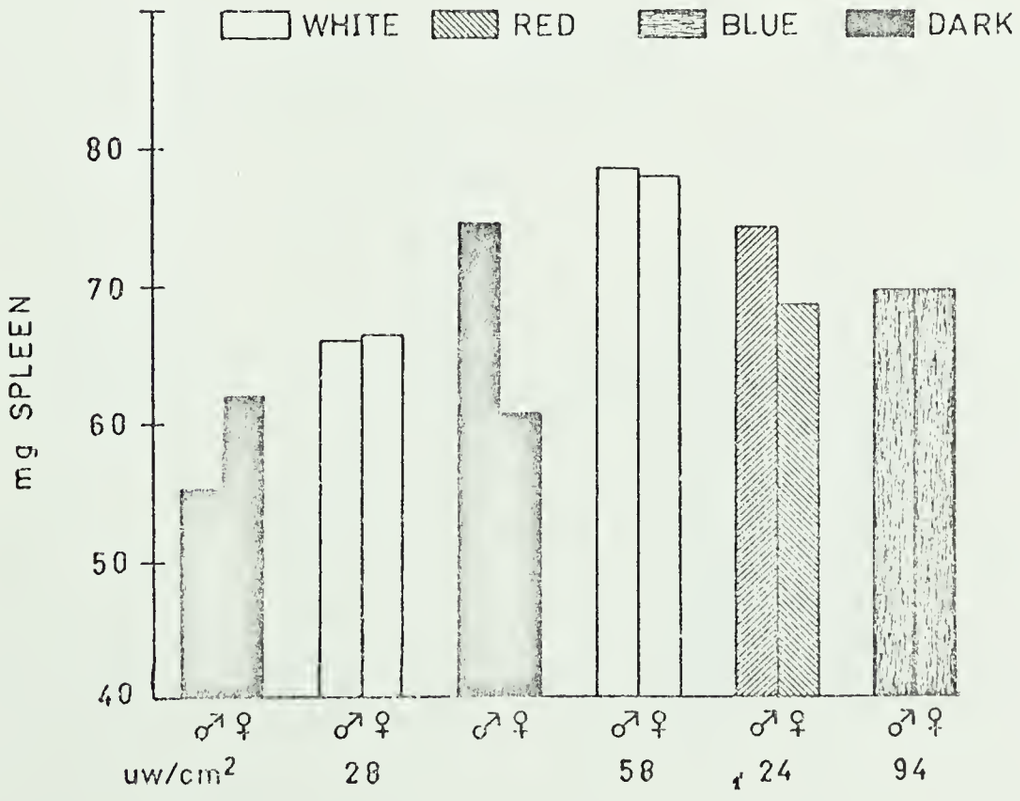
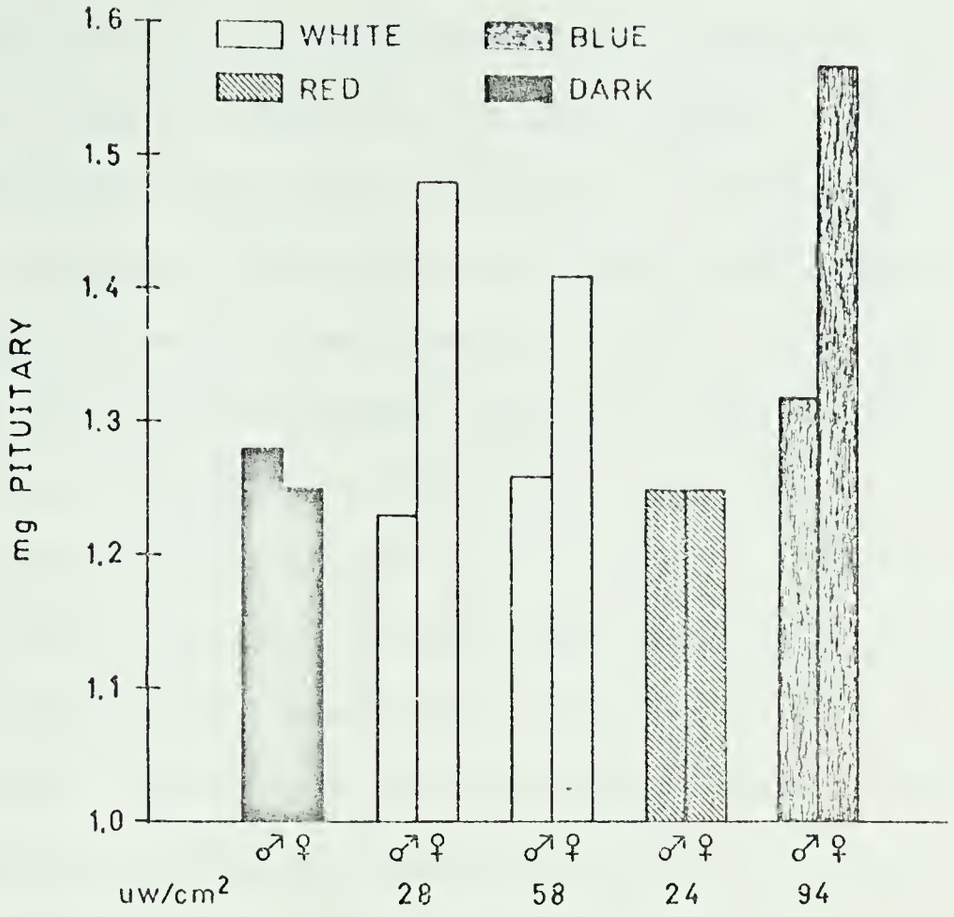
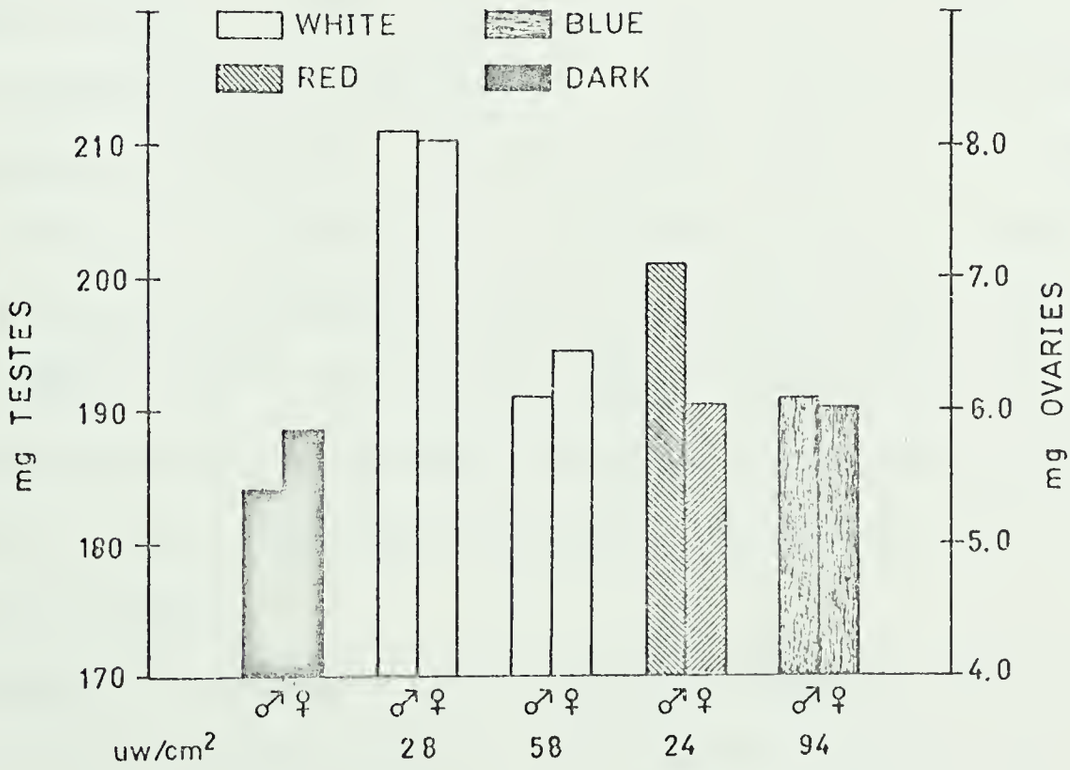

WHITE
RED
BLUE
DARK
mg SPLEEN
80
70
60
50
40
♂ ♀
uw/cm²
28
58
24
94

Figure 20. Pituitary weight of BALB/c mice under several light intensities. Age - 60 days.

Figure 21. Gonad weight of BALB/c mice under several light intensities. Age - 60 days.


WHITE
BLUE
RED
DARK
mg PITUITARY
1.6
1.5
1.4
1.3
1.2
1.1
1.0
♂ ♀
uw/cm²
28
58
24
94


WHITE
BLUE
RED
DARK
mg TESTES
210
200
190
180
170
mg OVARIES
8.0
7.0
6.0
5.0
4.0
♂ ♀
uw/cm²
28
58
24
94

Pituitary weights, although not significantly different between treatments, are shown for comparison with gonad weights. Gonad weights of mice reared under white light (28 $\mu w/cm^2$) were greater than of those reared in darkness. Gonad weights of animals under higher intensity white light (58 $\mu w/cm^2$), red light (24 $\mu w/cm^2$) or blue light (94 $\mu w/cm^2$) were lower than those of animals under white light of intensity 28 $\mu w/cm^2$ or in darkness. (See Table XV B for comparison of means.)

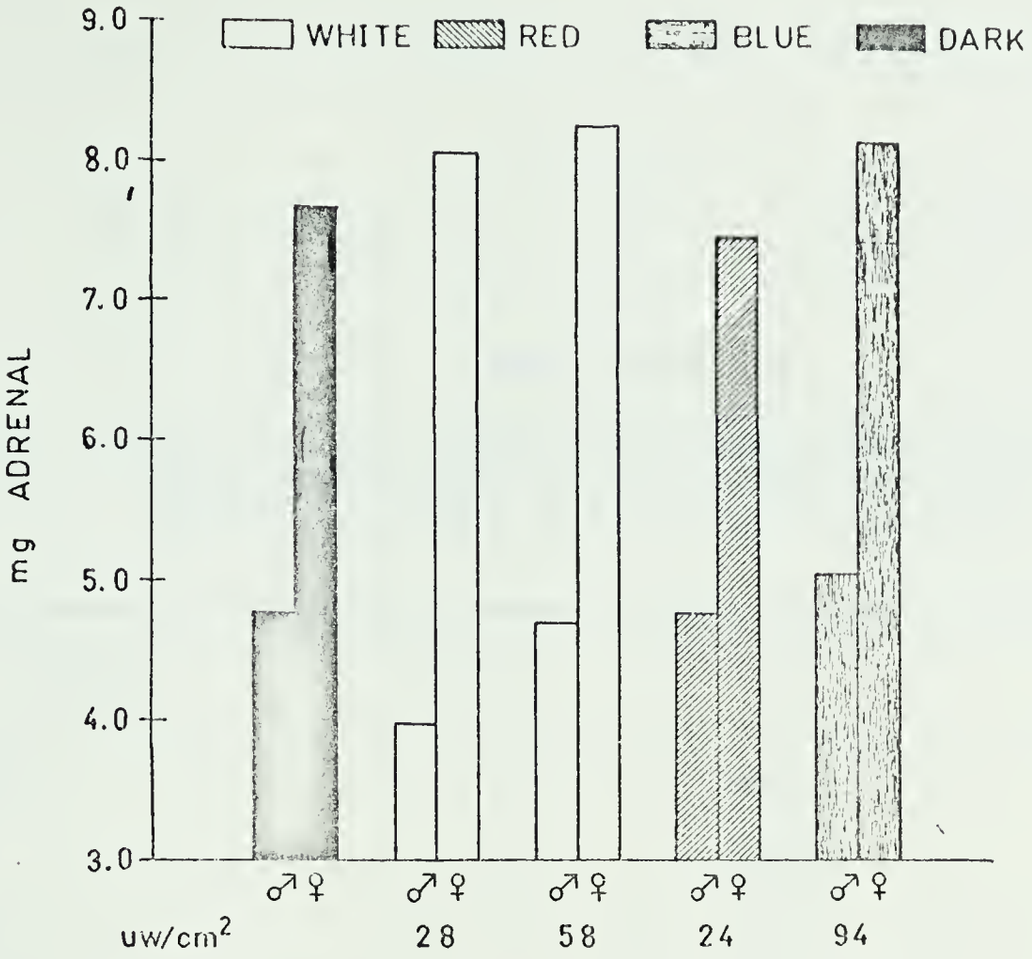
Figures 18 and 22 portray adrenal weights of C57/Bl and BALB/c mice. C57/Bl males reared in white light of intensity 58 $\mu w/cm^2$ had significantly heavier adrenals than males from the other four light environments. BALB/c males reared under white light of intensity 28 $\mu w/cm^2$ had lower adrenal weights than animals in the other environments. (See Table XVI B for comparison of means.)

Spleen weights for the two strains are shown in Figures 19 and 23. Spleen weights of C57/Bl mice of both sexes were not significantly different between lighting treatments ($p < .05$). The greatest average spleen weights for both sexes were from animals reared in white light of intensity 58 $\mu w/cm^2$. (Treating the data for the two sexes together reveals a statistical difference between the two white light treatments.) Spleen weights of BALB/c mice in white light of either 28 or 58 $\mu w/cm^2$ were lower than those of dark-treated animals, animals under red light (24 $\mu w/cm^2$) or under blue light (94 $\mu w/cm^2$). (See Table XVII B for comparison of means.)

Analysis of variance (2 x 2 x 5 factorial design) revealed that light affected pituitary weight ($p < .001$), gonad weight ($p < .001$), adrenal weight ($p < .05$) and spleen weight ($p < .001$).

Figure 22. Adrenal weight of BALB/c mice under several light intensities. Age - 60 days.

Figure 23. Spleen weight of BALB/c mice under several light intensities. Age - 60 days.


WHITE
RED
BLUE
DARK
mg ADRENAL
9.0
8.0
7.0
6.0
5.0
4.0
3.0
♂ ♀
uw/cm²
28
58
24
94

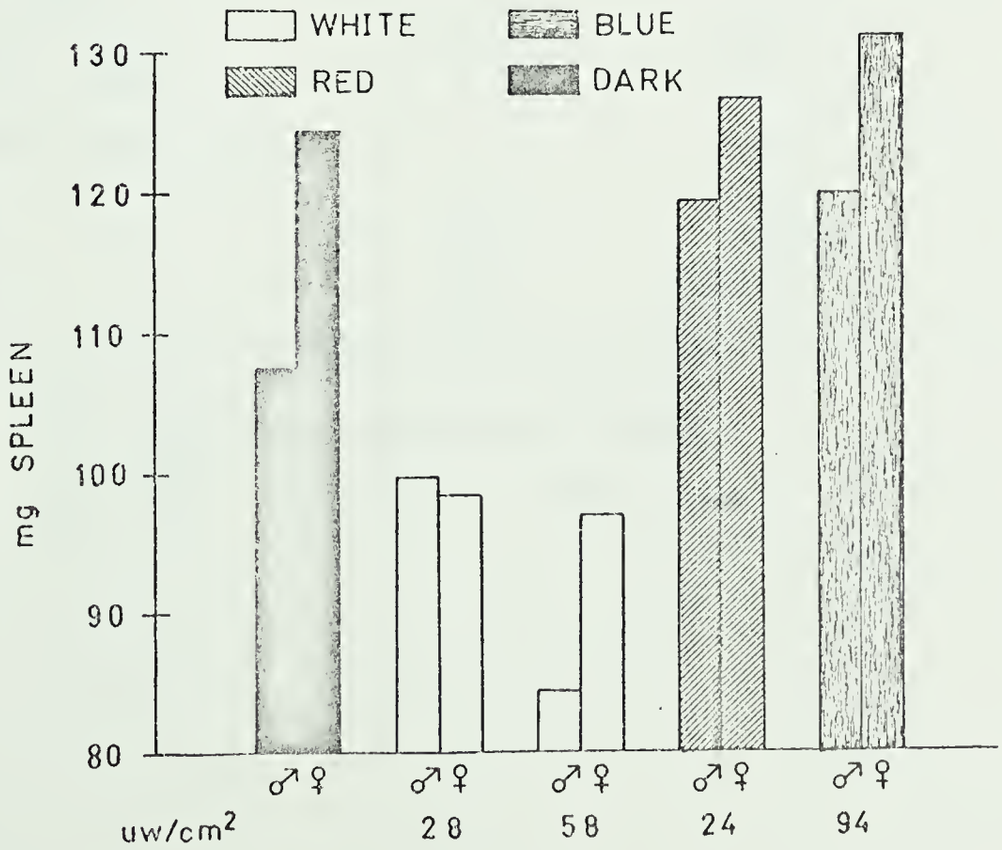

WHITE
RED
BLUE
DARK
mg SPLEEN
130
120
110
100
90
80
♂ ♀
uw/cm²
28
58
24
94

Table XIV A[†]

Effect of strain and sex on pituitary weight of mice under several light intensities (Experiment 7) Analysis of variance (2 x 2 x 5 factorial design)

Source	df	SS	MS
Strain	1	.32948	.32948***
Sex	1	.41474	.41474***
Light	4	.63836	.15959***
Strain x sex	1	.00176	.00176
Sex x light	4	.20138	.05035
Strain x light	4	1.0594	.26484***
Strain x sex x light	4	.17554	.04388
Error	80	2.0868	.02608
Total	99	4.9073	

[†]See Table B for comparison of means.

*p < .05 **p < .01 ***p < .001.

Table XIV B[†]

Effect of strain and sex on pituitary weight of mice under several light intensities (Experiment 7)

Light environment	Average pituitary weight (mg) (N = 5)			
	C57/Bl ♀	C57/Bl ♂	BALB/c ♀	BALB/c ♂
Dark	1.65	1.49	1.25	1.28
White (28 μw/cm^2)	1.85	1.54	1.48	1.23
White (58 μw/cm^2)	1.51	1.29	1.41	1.26
Blue (94 μw/cm^2)	1.18	1.24	1.57	1.32
Red (24 μw/cm^2)	1.38	1.31	1.25	1.25

Comparison of means

C57/Bl ♀

	1.38	1.51	1.65	1.85
1.18	NS	**	***	***
1.38	-	NS	*	***
1.51		-	NS	**
1.65			-	*

C57/Bl ♂

	1.29	1.31	1.49	1.54
1.24	-	NS	**	**
1.29		NS	*	**
1.31		-	*	**
1.49			-	NS

BALB/c ♀

F value NS at $p < .05$

BALB/c ♂

F value NS at $p < .05$

[†]See Table A for factorial analysis.

Table XV A[†]

Effect of strain and sex on gonad weight of mice under several light intensities (Experiment 7) Analysis of variance (2 x 2 x 5 factorial design)

Source	df	SS	MS
Strain	1	364.16	364.16
Sex	1	879782	879782***
Light	4	3348.5	837.12***
Strain x sex	1	65.885	65.885
Sex x light	4	2668.1	667.03**
Strain x light	4	1242.0	310.50
Strain x sex x light	4	1190.8	297.70
Error	80	10960	137.01
Total	99	899622	

[†]See Table B for comparison of means.

*p < .05 **p < .01 ***p < .001.

Table XV B[†]

Effect of strain and sex on gonad weight of mice under several light intensities (Experiment 7)

Light environment	Average gonad weight (mg) (N = 5)			
	C57/Bl ♀	C57/Bl ♂	BALB/c ♀	BALB/c ♂
Dark	4.55	198	5.84	184
White (28 μw/cm^2)	5.41	217	8.01	211
White (58 μw/cm^2)	4.20	173	6.44	191
Blue (94 μw/cm^2)	3.18	185	6.01	191
Red (24 μw/cm^2)	4.00	178	6.02	201

Comparison of means

C57/Bl ♀

	4.00	4.20	4.55	5.41
3.18	NS	*	**	***
4.00	-	NS	NS	**
4.20		-	NS	**
4.55			-	*

C57/Bl ♂

	178	185	198	217
173	NS	NS	NS	**
178	-	NS	NS	**
185		-	NS	*
198			-	NS

BALB/c ♀

	6.01	6.02	6.44	8.01
5.84	NS	NS	NS	*
6.01	-	NS	NS	*
6.02		-	NS	*
6.44			-	NS

BALB/c ♂

	191	191	201	211
185	NS	NS	NS	**
191	-	NS	NS	*
191		-	NS	*
201			-	NS

[†]See Table A for factorial analysis.

The effect of light on pituitary weight (Table XIV A) depended on strain ($p < .001$). The pituitary weight of BALB/c mice did not differ significantly ($p < .05$) between lighting treatments. The effect of light on pituitary weight did not depend on sex.

The effect of light on gonad weight (Table XV A) was dependent on sex ($p < .01$) but not on strain ($p < .05$); both sexes, of either strain, showed significant differences between some of the lighting treatments (Table XV B).

The effect of light on adrenal weight (Table XVI A) was not directly dependent on either sex or strain. Factorial analysis of variance, however, revealed a three-way interaction of sex by strain by light ($p < .01$). Significant differences in mean adrenal weights of mice from either strain under different lighting treatments could only be demonstrated in males (Table XVI B).

Table XVI A[†]

Effect of strain and sex on adrenal weight of mice under several light intensities (Experiment 7) Analysis of variance (2 x 2 x 5 factorial design)

Source	df	SS	MS
Strain	1	54.997	54.997***
Sex	1	119.81	119.81***
Light	4	3.3572	.83931*
Strain x sex	1	28.879	28.879***
Sex x light	4	2.1904	.54762
Strain x light	4	2.2759	.569
Strain x sex x light	4	4.7968	1.1992**
Error	80	24.114	.30143
Total	99	240.43	

[†]See Table B for comparison of means.

*p < .05 **p < .01 ***p < .001.

Table XVI B†

Effect of strain and sex on adrenal weight of mice under several light intensities (Experiment 7)

Light environment	Average adrenal weight (mg) (N = 5)			
	C57/Bl ♀	C57/Bl ♂	BALB/c ♀	BALB/c ♂
Dark	5.67	4.24	7.68	4.78
White (28 μw/cm^2)	5.25	3.89	8.06	3.98
White (58 μw/cm^2)	5.27	5.18	8.25	4.70
Blue (94 μw/cm^2)	5.01	4.02	8.13	5.04
Red (24 μw/cm^2)	5.61	3.91	7.48	4.78

Comparison of means

C57/Bl ♀		C57/Bl ♂			
F value NS at p < .05		3.91	4.02	4.24	5.18
	3.89	NS	NS	NS	***
	3.91	-	NS	NS	***
	4.02		-	NS	***
	4.24			-	***
BALB/c ♀		**BALB/c ♂**			
F value NS at p < .05		4.70	4.78	4.78	5.04
	3.98	NS	**	**	***
	4.70	-	NS	NS	NS
	4.78		-	NS	NS
	4.78			-	NS

†See Table A for factorial analysis.

Table XVII A[†]

Effect of strain and sex on spleen weight of mice under several light intensities (Experiment 7)
Analysis of variance (2 x 2 x 5 factorial design)

Source	df	SS	MS
Strain	1	40320	40320 ***
Sex	1	184.96	184.96
Light	4	3965.9	991.48***
Strain x sex	1	1089.0	1089.0*
Sex x light	4	181.14	45.285
Strain x light	4	6328.1	1582.0***
Strain x sex x light	4	672.30	168.07
Error	80	16585	207.31
Total	99	69327	

[†]See Table B for comparison of means.

*p < .05 **p < .01 ***p < .001.

Table XVII B[†]

Effect of strain and sex on spleen weight of mice under several light intensities (Experiment 7)

Light environment	Average spleen weight (mg) (N = 5)			
	C57/B1 ♀	C57/B1 ♂	BALB/c ♀	BALB/c ♂
Dark	60.8	74.6	124.2	107.4
White (28 μw/cm^2)	66.4	66.0	98.2	99.8
White (58 μw/cm^2)	78.0	78.4	97.0	84.2
Blue (94 μw/cm^2)	69.8	69.8	131.2	120.0
Red (24 μw/cm^2)	68.6	74.2	126.8	119.4

Comparison of means

C57/B1 ♀

F value NS at $p < .05$

	BALB/c ♀ 98.2	124.2	126.8	131.2
97.0	NS	NS	*	*
98.2	-	NS	*	*
124.2		-	NS	NS
126.8			-	NS

C57/B1 ♂

F value NS at $p < .05$

	BALB/c ♂ 99.8	107.4	119.4	120
84.2	NS	*	**	**
99.8	-	NS	NS	NS
107.4		-	NS	NS
119.4			-	NS

[†]See Table A for factorial analysis.

DISCUSSION

In 1932 Hohlweg and Junkman recognized the central nervous system as regulator of pituitary gonadotrophic function; they presented for the first time the cybernetic unit including central nervous system, pituitary and gonads. This model was essential for an understanding of experiments on the effects of light on reproductive function in birds and mammals. Extensive research has been done on the location of the photoreceptor for reproductive system responses and the neural pathways involved (see Introduction). Although Benoit (1935a) has shown that the eye region of the duck is involved in the gonadal response to light, there is no clear evidence that the retina itself is the receptor for this response. Menaker *et al.* (1970) have shown that the retina is not necessary for the gonadal response of the sparrow to light. Experiment 5 suggests that in deer mice, as in the ferret (Clark *et al.*, 1939), the rat (Browman, 1942) and hamster (Reiter, 1969) the eye is involved in photo-neuro-endocrine responses to light. An intact pituitary is also necessary for the photosexual response to light of the ferret (Hill and Parks, 1933) and of the duck (Benoit and Assenmacher, 1959).

It appears from the literature, as well as from the results of this study, that light acts in a more fundamental way than as a synchronizer of reproductive events. Pierpaoli *et al.* (1970)have pointed out that a fundamental distinction must be made between the action of a hormone on the mature target organ and the action of a hormone in the differentiation of target organs and tissues prepared by their genetic programme for a hormonal action to take place. This has led him to

distinguish between the action of developmental hormones and the action of hormones of the mature endocrine system. It follows that a distinction must be made between the effects of light on events associated with maturation of the hypothalamus-pituitary-target-organ axis and the effects of light on the fully functional control unit involving central nervous system, pituitary and target organs. In this context, the present study had dealt mainly with the period of rapid maturation around the time of puberty. The findings are consistent with the view that the endocrine events of this period are fundamentally different from those of the later "maintenance" period.

The present study suggests that deer mice, as well as C57/B1 and BALB/c mice, are photosensitive. Light deprivation delays onset of puberty (low body weight, small gonad weight, delay of vaginal introitus) in deer mice. This confirms observations in rats (Luce-Clausen and Brown, 1939; Fiske, 1939). In addition, the degree of advancement of puberty by light appears to be intensity-dependent. Williams (1969) has observed that the age at first estrus in C57/B1 mice is also dependent on the intensity of light under which mice are reared. The present study confirmed that the endocrine system of C57/B1 mice is very sensitive to the intensity of light during rearing. Pituitary, gonad, adrenal and spleen weights varied with the light intensity under which the mice were maintained.

Gonad weights of deer mice reared under red light were greater than those of deer mice reared under blue or green light. This agrees with earlier studies on ducks (Benoit, 1964), chickens (Foss and Arnold, 1969) and other birds (see Introduction).

Accurate specification of the attributes of light is essential so

that results of such light experiments can be evaluated. To investigate the effects of light intensity on a physiological parameter almost any unit of measurement is suitable. However, this is not so for wavelength studies. Luminosity criteria have been used in a number of action spectrum studies, some of which were designed to provide environments of approximately equal luminous intensity (see Introduction). Luminosity measures, however, are unsuitable for action spectrum studies since luminosity units (lumens, lux or foot-candles) express light intensities in terms of the sensation evoked in the average human observer. The greatest sensitivity of the light-adapted human eye to light energy is at 555 nm. The receptor mechanisms involved in photo-neuro-endocrine responses are not necessarily the same as those involved in vision, nor are the receptor(s) and spectral sensitivities of all vertebrates necessarily the same as for humans. The purpose of action spectrum studies is to characterize unique spectral influenceson distinct physiological responses. Early investigators who have not taken into consideration the nature of luminosity units of measurement may have inadvertently designed a considerable amount of error into their wavelength experiments. At equal radiant energy green light has a much greater luminous intensity than blue or red light (see Materials and Methods). A number of action spectrum studies on the gonadal response of birds to light have taken this into consideration. Hollwich and Tilgner (1961), Benoit and Assenmacher (1966) and Foss and Arnold (1969) have designed action spectrum studies to provide coloured environments of equal radiant energy.

An additional factor, however, must be taken into consideration when specifying the attributes of light in an action spectrum study.

Light is basically a quantum phenomenon. At equal energy levels the long wavelengths are characterized by a greater number of quanta than the short wavelengths. The energy of a photon is inversely related to wavelength according to the equation:

$$E = hc/\lambda$$

where E is the energy of the photon, h is Planck's constant and λ is the wavelength. Thus the energy of a given number of quanta of short wavelength light is greater than the energy of the same number of quanta at long wavelength. In spectral environments of equal radiant energy, the number of quanta increases with wavelength: red light provides a greater number than does blue light of equal energy. The results of Experiment 1 suggest that the greater gonadal response, as well as the response of the spleen, of deer mice to light of long wavelengths may be due to the fact that at equal energy, light of long wavelengths provides more quanta. When the number of quanta in blue and red environments were equated, the differential response to wavelength was lost (Experiment 3). Testis weight was significantly greater in animals reared under light of greater number of quanta irrespective of wavelength. Similarly spleen weights in both sexes were significantly greater in animals reared under coloured light of greater number of quanta irrespective of wavelength. This suggests that changes in organ weights of deer mice are related to the number of quanta of light rather than to energy level or wavelength *per se*. Since all wavelengths are effective in stimulating gonadal growth it can be assumed that the photoreceptor for non-visual neuro-endocrine responses is sensitive to the complete visible spectrum.

Further investigation is necessary to determine the photoreceptor pigments involved in such photoresponses.

Considerable variation was observed in organ weights of deer mice both within and between lighting treatments, suggesting that the light response may have been tempered by genetic factors. The study of the effects of light on C57/Bl and BALB/c mice confirmed this. Factorial analysis of variance revealed that variations of pituitary and spleen weights with light intensities were significantly dependent on strain (Tables XIV A, XVII A).

The effects of light on mice of inbred strains and deer mice from a natural population were not the same. In their natural environment only some deer mice born of the first spring litter reach sexual maturity and breed in the year of their birth (Fuller, 1969). In the present study only a few deer mice reared under bright light had reached puberty (as indicated by vaginal introitus) at 60 days of age. Pituitary weight, as well as body weight, relative ovary weight, relative testis weight and relative spleen weight increased with intensity of light under which animals were reared. Mice of the C57/Bl and BALB/c strains reach sexual maturity much earlier. At 60 days of age all mice had reached puberty (all females had perforate vagina) regardless of light environment. Experiment 7 thus is a study of post-puberal mice. Body weight (see Appendix A) was not significantly different between groups of mice reared in different light environments. Organ weights varied with light independently of body weight. Experiment 7 suggests that the pituitary-gonadal response of C57/Bl and BALB/c mice to light is intensity-dependent. Gonad weight was greater in white light of intensity 24 $\mu w/cm^2$

(conventional laboratory light) than in darkness. Under higher intensities these mice showed a decrease in gonad weight. Parallel changes occurred in pituitary weight of C57/Bl mice. The depression of gonad weight by light of high intensity is similar to that observed in rats under constant light. Although constant light accelerates the onset of puberty, with associated body weight and gonad weight increases, in post-puberal female rats under constant light there is a decrease in ovary weight (Fiske, 1939). Thorpe (1967) similarly has demonstrated that light affects young ferrets differently from adult ferrets. Adult ferrets become anoestrous on exposure to short photoperiods. The same short photoperiod will induce early estrus in young ferrets. Critchlow (1963) suggests that FSH inhibition by estrogen is the cause of the phenomenon in post-puberal female rats, and compares the mechanism to that involved in the refractory periods of passerine birds (see Farner, 1959, for review), ferrets and mink. If testicular weight changes can be taken as a reliable index of plasma FSH levels, as Fraschini and Martini (1970) suggest, then the reduced gonadal weight found in C57/Bl and BALB/c mice under high intensity light may indicate low FSH. A study of adult deer mice, preferably from an inbred colony, would be necessary to determine whether a similar phenomenon occurs in these animals.

The influence of number of animals per cage is also different for deer mice and C57/Bl mice. Bronson and Eleftheriou (1963) have reported that adrenal weight of C57/Bl mice (age 60 to 70 days) increases with increase in density from 1 to 8 animals per cage but under similar conditions adrenal weight of deer mice showed no difference. Experiment 4

suggests that a high density of animals inhibits maturation of the endocrine system. Pituitary, testis, adrenal and spleen weights of animals reared at a density of 10 to 12 animals per cage were lower than organ weights of animals from a control group. The effect was somewhat less when a similar group of animals were reared in a higher intensity light environment. In the natural environment crowding of up to 28 deer mice per nest in winter has been reported (Stebbins, 1971). Crowding is likely one of the factors which inhibit breeding of deer mice during the winter. Darkness is possibly another.

It appears that males and females of the C57/Bl and BALB/c strains are equally photosensitive. Variation in pituitary weights with light environment was not significantly dependent on sex (see Table XIV A). Sex differences observed in the gonadal response to light (Table XV A) may be due to sex differences in gonadotrophin secretion. An alternative explanation is that the sex differences observed are due to differential sensitivity of ovaries and testes to gonadotrophins.

Light also affects adrenal weight in deer mice, C57/Bl and BALB/c mice. Relative adrenal weights of deer mice reared under dim light were significantly greater than those of deer mice reared under constant darkness. Adrenal weight appears to be related in a complex way to the intensity of light under which the mouse is reared. Relative adrenal weights of deer mice reared under bright light were significantly lower than those of animals reared under dim light. Light may accelerate the onset of age-related involution of the adrenal.

In most mice adrenal weight is greater in females (Sakiz, 1960) and such is the case in deer mice, C57/Bl and BALB/c mice. Badr and

Spickett (1971) have observed that the magnitude of this sex difference is at least in part genetically determined in A/Cam, CBA/FaCam and SF/Cam strains. Analysis of variance (Table XVI A) reveals this is probably so for C57/Bl and BALB/c mice as well.

Badr and Spickett (1970) suggest that there may also be strain differences in those aspects of adrenal structure which distinguish females from males. In both male and female mice there is a juxta-medullary X-zone which involutes under the influence of androgen in the male around the time of puberty and disappears in the female somewhat later. In male BALB/c mice of 45 days of age this transitory zone has disappeared, but in females of the same strain the X-zone is well developed at this time (Sakiz, 1960). If females of this strain are injected with testosterone the X-zone disappears prematurely and adrenal weight and adrenal corticosterone levels are reduced to levels observed in the male (Sakiz, 1960). Thus there appear to be both strain and sex differences in sensitivity of the X-zone to steroids involved in this age-related involution. The strain by sex by light interaction observed in the analyses of adrenal weights of C57/Bl and BALB/c mice (Table XVI A) can be understood by taking these factors into consideration. Light may be thought of as providing a stimulus to the pituitary-gonadal axis. The gonadal response in turn apparently affects the timing of involution of the adrenal X-zone, a normal event of maturation. However, analysis of variance of the data (Table XVI A) cautions against concluding that this is the only factor involved. Reports in the literature suggest that other factors may be involved as well. Sex steroids have a moderating influence on adrenal weights throughout the life of the rat (Fiske, 1962).

Furthermore, in the female rat, light affects the pituitary-adrenal axis in the absence of the ovary (Critchlow, 1963). Kitay (1961) has found that the female rat adrenal is more sensitive to stimulation by ACTH than the male rat adrenal.

If light has an effect on activity of the pituitary-adrenal axis, a possibility to be considered is that an unnatural light environment may act as a stressor on the mouse (a stressor as understood in the Selye hypothesis, eliciting an increase in adrenal weight, decrease in eosinophil count and breakdown of lymphoid tissue). However in deer mice reared under bright light, adrenal weight was lower than in dim light, eosinophil count was higher (Table VI, Figure 11) and spleen weight greater. Rather than acting as a stressor increasing light intensity seems to be stimulating normal processes of maturation.

When deer mice were gonadectomized (Experiment 6) thymus weight was greater in male mice under dim light than in male mice under bright light ($p < .05$). Thymus enlargement occurs after gonadectomy in many species (see Dougherty, 1952, for review) and such is the case with male deer mice (Table XII) reared under dim light. Thymus atrophy has often been taken as an indicator of pituitary-adrenal mediated stress (Thiessen and Rodgers, 1961). Thymus weight of gonadectomized deer mice under bright light was not significantly different from unoperated or sham-operated litter mate controls. Although the difference in thymus weight of castrated animals in the two light environments is interesting, the data on thymus weight in Experiment 6 again fail to support the view that increasing light intensity acts as a stressor.

Several investigators have found that both gonadal hormones and

adrenocortical hormones have a moderating influence on lymphatic tissue throughout life (see Dougherty, 1952). Although this may be true, it is interesting that there were no sex differences in the spleen weight response to light of inbred mice (Table XVII A) or deer mice (Table VII, Table X). Spleen weight of deer mice increased with intensity of light under which the animals were reared. Spleen weight of C57/Bl mice under light of intensity 58 $\mu w/cm^2$ was greater than spleen weight of C57/Bl mice under light of intensity 28 $\mu w/cm^2$. Spleen weight of BALB/c mice in white light of either 28 or 58 $\mu w/cm^2$ was lower than that of dark-treated animals, animals under light of intensity 24 $\mu w/cm^2$ (red) or animals under light of intensity 94 $\mu w/cm^2$ (blue) (Figures 19 and 23). Interpretation of the differences in spleen weights of the two inbred strains under different light conditions must take into consideration that there is a normal age-involution of most lymphoid tissue around the time of puberty, although the spleen continues to increase in weight. Since increasing light intensity accelerates the onset of puberty it is not surprising to find, for example, in BALB/c mice a lower spleen weight in light of intensity 58 $\mu w/cm^2$ than in darkness and a higher spleen weight in light of intensity 94 $\mu w/cm^2$.

Very little is known about the differential sensitivity of tissues to developmental hormones such as STH and sex steroids, although light effects of sexual maturation have been well documented. Light also influences STH secretion. Blinded rats have reduced pituitary and plasma levels of growth hormone (Sorrentino *et al.*, 1971).

Growth hormone has been viewed as a "thymotrophic" hormone (Pierpaoli, 1970) in that it increases immunocompetence of thymus lymphocytes and thymus-derived lymphocytes in the spleen (during maturation of

the immune system cells migrate from thymus to spleen). There is considerable evidence that immunological maturation depends on endocrine function; the most critical effect of hormones, Pierpaoli suggests, is during the development of the immune system.

It is interesting that STH administered to adult rats (Moon *et al.*, 1950) or mice (Szego and White, 1949) had no effect on spleen weight but that prolonged STH treatment of hypophysectomized rats (Moon *et al.*, 1951) resulted in enlarged thymus, spleen and lymph nodes. It seems likely that the spleen weight response of C57/Bl, BALB/c and deer mice is mediated at least in part by growth hormone.

It appears that a theory of maturation involving the central nervous system, pituitary and target organs is necessary to suitably explain the observed variations in organ weights of animals reared under different lighting conditions. Light appears to accelerate the onset of developmental events mediated by pituitary-target organ hormones, events which occur according to the genetic programme of development. This is particularly obvious at the time of puberty. This work has brought forth no evidence that increasing light intensity acts as a stressor as described in the Selye hypothesis. Rather, it appears that light acts as a stimulus which accelerates normal development.

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APPENDIX

APPENDIX A

Body weight of mice of inbred strains
Analysis of variance (2 x 2 x 5 factorial design)

Source	df	SS	MS
Strain	1	32.948	32.948***
Sex	1	295.84	295.84***
Light	4	8.1686	2.0421
Strain x sex	1	3.3124	3.3124
Sex x light	4	20.821	5.2052
Strain x light	4	18.291	4.5728
Strain x sex x light	4	14.513	3.6281
Error	80	205.37	2.5671
Total	99	599.26	

***$p < .001$.

APPENDIX A

Body weight of mice of inbred strains

Light environment	Average body weight (gm) (N = 5)			
	C57/B1 ♀	C57/B1 ♂	BALB/c ♀	BALB/c ♂
Dark	21.10	22.54	21.70	24.66
White (28 $\mu w/cm^2$)	19.24	25.40	21.82	25.20
White (58 $\mu w/cm^2$)	20.54	24.50	20.28	24.04
Blue (94 $\mu w/cm^2$)	19.70	23.48	21.02	24.66
Red (24 $\mu w/cm^2$)	19.10	22.78	22.42	24.06

APPENDIX B

Organ weights of C57/Bl mice under constant darkness*

	C57/Bl ♀ (N = 5)	C57/Bl ♂ (N = 5)
Body weight	19.5 gm	25.8 gm
Pituitary	1.62 mg	1.57 mg
Gonad	4.81 mg	1.96 mg
Adrenal	4.93 mg	3.89 mg
Spleen	61.4 mg	56.0 mg

*Organ weight data are also presented in Figures 16 to 19.